Ethology practical

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Made in the project entitled "E-learning scientific content development in ELTE TTK" with number TÁMOP-4.1.2.A/1-11/1-2011-0073. Consortium leader: Eötvös Loránd University, Consortium Members: ELTE Faculties of Science Student Foundation, ITStudy Hungary Ltd.

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Chapter I. Field ethology – Conducting behavioral observations in the Budapest Zoo

Péter Pongrácz

1. OBJECTIVES

This practical is about how to conduct basic observational studies on free moving animals. Students have to use their knowledge about the theory of ethological description of behaviour, the concept of ethogram and the most widely used methods of behavioural research. In the Budapest Zoo the students participate on a brief theoretical introduction, and then they can choose their subjects freely from among the numerous species available in the Zoo. The practical offers a wide array of possible methods and research questions, as each species shows more or less different behaviour and activity patterns.

The main goal of this practical is to provide a model of a full ethological study, beginning with the pilot observations and asking the proper research question, following with the data collection and analysis, and ending with writing a report and drawing conclusions from the results.

2.INTRODUCTION

2.1 The design of a scientific research study

Data collection is not the first step of the scientific research. Ideally, each study (which can either be observational or experimental) starts with establishing a proper research paradigm, or more simply, a research question. A new study's question can be derived from a previous research work, but it can also originate from an accidental or planned observation or pilot study as well.

The first 'official' step of an investigation is forming the research question. The next step is to choose or develop suitable methods, which will enable us to collect the necessary data to answer the question. For choosing the appropriate method one will need to know well enough the circumstances and the subjects of the study, and it is also very useful if we know some of the related researches, which were published earlier on the similar topic. In other words, the methodological planning of a study requires a throughout practical and theoretical knowledge from the scientists.

Knowing our question and the outline of the methods we will use for answering it, it is worth to enumerate those hypotheses, which are basically the possible outcomes (answers) of the research. Depending on the planned research, there are minimum two, but sometimes three or more theoretical answers that can exist. A preliminary analysis of these hypotheses can provide considerable help for the future, when we already have the results of our investigation.

This practical differs from the others along the course, where the students join to the experimental work *after* the previously mentioned three steps of research planning. Our main goal here is to provide the students a chance to conduct a full research right from the first step. They have to discover an interesting phenomenon, form a suitable research question, plan the proper data collecting methodology, and execute the observation. In the next paragraphs we provide a brief theoretical background about how to describe animal behaviour scientifically, and what kind of basic ethological procedures are available for conducting an observational research.

2.2 Components of behaviour

According to one of the main principles of ethology, behavior is a process that is dividable to discrete **behavioural elements**. These can be non-overlapping, for example a hen cannot sit and stand at the same time. Other behavi-

oural elements can overlap, for example the hen can peck while it is standing. The fact that a continuous string of behaviour can be divided to elements had a very important part in forming modern ethological thinking. Natural sciences require exact measuring methods, and this in turn would be impossible without unambiguous definitions and repeatable experiments. Knowing a few basic definitions are also necessary for the successful participation on this practical.

Behavioural elements are the smallest building blocks of behaviour, which are usually uniform among the members of a species. It is important to note however that behavioural elements can change along the life of an animal, for example because of ontogenetic reasons, environmental effects or learning. Still, it remains true that a particular behavioural element remains the same if we compare many individuals of a species, which are of the same age (and sometimes same sex). **Behavioural units** are such well-outlined sequences of behaviour, which consist of two or more behavioural elements, which usually belong together on the basis of functional reasons. The preening of birds is a good example for behavioural units. Preening can involve several behavioural elements, like fluffing up the feathers, manipulating them with the beak of the bird, pushing out the secretion of the coccygeal gland, smearing it on the feathers etc. **The ultimate and full collection of all behavioural units of a species is the ethogram**. Ethograms contain usually independent (non-overlapping) behavioural units, which are described with as few functional terms as possible. Favoring formal descriptions over the functional ones is preferred because subjectivity can plague the functional evaluations, leading with this to misinterpretations of the behaviour.

The term ‘measurement’ is quite easy to understand in such fields of biology, like genetics, physiology or biochemistry. However, at first glance behaviour is very hard to turn something that is ‘measurable’. Introducing behavioural elements provided considerable help to decide “what to measure”. From this point behaviour became a quantitative phenomenon, providing numerical data, which is possible to be analyzed with statistical methods.

Right at the beginning we should decide what kind of behavioural elements we will collect and from which purpose. Obviously it depends on many factors, for example the available knowledge about the subjects. If the research is about some kind of original, descriptive study of a species or phenomenon, a finely detailed, throughout collection of behavioral elements may be necessary. At the other hand, if our subjects are members of a well-studied species, and we are seeking the answer for a specific, narrowly defined question, a few, or maybe one behavioural element may be sufficient to be observed during the investigation.

When studying behaviour, it makes a big difference when using **formal or functional description**. Formal description is the most objective way of ethological observations, because it does not involve any of the observer’s personal opinions, assumptions, which for example could be influenced by his/her previous experiences. Formal description consists of usually very simple elements, like the animal stands, sits, looks to the right/left, reaches with its neck etc. Collecting data about the space usage of animals belongs also to the formal description – like when the observer makes notes about where is the animal (or group of animals), or is the animal near or far from something.

Formal descriptions are time consuming and labour intensive, therefore if we have enough knowledge about the subjects and their behaviour (based on the literature or on our preceding results), we can opt for the **functional description** of behaviour. In this case we observe and collect such units of the behaviour, which can be characterized with a well defined function. Such units can be for example when an animal eats, drinks, plays, fights, collects nest material etc. Functional behavioural units can consist of several formal elements, for example ‘fight’ may involve many distinct movements – each of these can be defined, collected and counted. Functional description can be regarded as a compression, or simplification of the observation, which, in turn, makes the job of the ethologist much easier. Obviously, such a simplification does not come without drawbacks. It can happen that during a functional labeling some important details will be lost from the behavioural description. For example when we use a functional category “eats the meat” for describing the behaviour of wolves, this will not show the difference between the behavior of a dominant and a subordinate animal. The dominant wolf may approach the quarry in an upright posture, meanwhile the subordinate wolf sneaks there almost flattened on the ground. Another mistake is when somebody includes a not well grounded opinion to the functional description. For example if the observer does not know that flamingos feed only in the water, can label as “feeding” a behavior when the birds move their beaks in the grass on the shore. This labeling will be obviously false.

Researchers on the Department of Ethology of Eötvös Loránd University developed a combination of the formal and functional behavior coding, using the paradise fish (*Macropodus opercularis*) as a model (Csányi, 1985; Csányi et al., 1985). Each behavioral element was described with the help of the following three parameters:

Posture – what does the animal do?

Location – where is the animal?

Orientation – which part of the environment (living or not) the behaviour is directed at?

This kind of coding can provide such further details about the behaviour, which would not be possible with the traditional formal description. At the same time it retains the objectivity through the formal elements it uses, therefore we can avoid such false assumptions that can be the consequence of a purely functional description (for example interpreting the fish' behavior as "asking for food" when they are approaching that part of the aquarium where a human appeared).

2.3 The subjects of an ethological study

In the simplest case the researcher has only one subject at a time that he/she has to follow. However, it often happens that there is a smaller or larger group of animals on the scene. In this case we have to decide how many of them will be observed, and which ones will we observe. We have to be able to give a reason for our choice, why did we choose particular subjects and why did we exclude the others. Another, often difficult task is the identification of particular subjects from a larger group, many times with considerable delay after the initial observations.

Choosing the subjects is a matter of the planned study. In other words, our research question usually determines which subjects we will need. If we are interested in the behaviour on the group level, obviously more than one individual should be observed simultaneously. In other times a larger group represents rather only a disturbing factor, because our question is aimed to the behaviour of a particular individual, or some kind of well defined sub-groups of the many available subjects. As a basic rule we should always remember to identify reliably the individuals, when our research is about the behaviour of non-randomly chosen subjects.

In general, we can conduct focal or group-level observations. During a **focal observation** we always concentrate on one specific individual (or a specific pair of subjects etc.). After identifying our focal subject, we basically ignore the others' behaviour. When conducting a **group-level observation**, we have to collect data about each subject that is present at a time.

2.4 How can we collect behavioural data?

We have already figured it out, which kind of behavioral elements will we collect, and we have also chosen our subjects for observation – but we still have to decide upon a very important detail: what exactly will we *do*? When talking about behavioural coding, the method of sampling basically means what kind of details of the behaviour and how often will we collect. A good start is if we base our choice of sampling method on the initial research question – as this method should be suitable for collecting the data that can be used to formulate the answer.

As any animal's behaviour is an unbroken string of simpler elements, a logical choice for behavioural research is the **continuous observation**. The observer should not loose from his/her scope the subject (subjects) during the observational period, and each behavioural element should be recorded in that order as they follow each other. Along with this usually a time dimension is also recorded, showing the duration of the behavioral elements. Continuous observation can be very useful during initial exploratory studies, when the subjects are less known, or when the behaviour is very complex (for example courtship, ritualized fight, nest building etc.). At the same time continuous observation has its drawbacks, too. It would be near impossible to follow and record simultaneously the behaviour of all members of a larger group. In other cases the researchers are interested in the occurrence of one or only a few specific behavioural elements (for example, alarm calls when predators appear). It would be unnecessary and very time consuming to record everything else what the subjects are doing in this case. When the continuous observation is not an option or when it is unnecessarily work-needy, it is better to use some form of sampling of the behaviour.

When opting for a **sampling method**, we should decide the time and space boundaries of the data collecting. **Space** can be an important parameter, when we record the spatial distribution or movement of the subjects. We can use natural categories of space (like the animals can be on the tree, or down at the ground level), or we can divide the space artificially to (usually equal) sections. Using space as a sampling aid, we can count for example, how many subjects are using a particular area, or what are the subjects doing at a particular place, or when did a particular subject enter a specific part of the observational area. **Time** is another important factor of sampling. Usually we employ equal periods for recording the behaviour. For example one can record in every 20 s the actual behaviour

of the subjects, or count how many subjects are doing a particular behaviour. A special variant of sampling is the **behaviour-based coding**. When opting for this, we are interested in the occurrence of only one (or two, etc.) specific behavioural element or unit, and independently of its spatial or temporal distribution, we record it (for example, the number of mountings among the baboons). This kind of sampling method is used when we are interested in a behaviour that is occurring only seldom, or very irregularly. When a behaviour is fairly common, is better to opt for the time-interval based sampling.

Another method for recording a small, limited number of behaviors is the **'yes-no' sampling**. It is usually connected to time-interval based data collection. In this case we record for example the number of animals showing or not showing a particular behaviour in a repeated time schedule. A related method is when we record the spatial distribution of the subjects repeatedly, for example on different places of the area.

3. MATERIALS

3.1 Location

Students can access the full area of the Budapest Zoo, including the indoor locations, too. As this practice can happen as a courtesy of the Zoo and the students and their demonstrator are the guests of the Zoo during the practical, it is very important that the students should adhere themselves to the current regulations of our host. Observations can be conducted to the closing time of the Zoo from the beginning of the practical (at least 3-4 hours, depending on the actual timetable of the semester that sets the start of the practical). Especially when the practical is afternoon, it is worth to keep in mind that the indoor facilities of the Zoo usually close 30 min earlier than the Zoo itself.

3.2 Subjects

Students can choose freely the subjects from among the Zoo's inhabitants. Beside the animals, there is an option for human ethological observations, too. With proper research question (and keeping in mind that the observation must not disturb the visitors), the behaviour of the Zoo's visitors can also be recorded.

For choosing of subjects, a basic note should be remembered: in general only active subjects will provide relevant and well-collectable data. Animals that do not or barely move for longer periods of time are not really suitable for the purposes of the ethology practical. At the same time the very vigorous activity can cause difficulties, too. Especially when we observe several subjects, or try to follow a focal animal among many others, the behaviour of the fast moving subjects are not the easiest to describe. (The same is true for identifying particular animals in a highly active group.)

Before we decide, which animals will we observe, it is recommended to do a throughout walk around in the Zoo. During this we should look for interestingly behaving, active animals, because this helps to formulate a good research question. Obviously, another possibility if somebody arrives with an a priori prepared research plan and target species. However, we emphasize that it is absolutely suitable and recommended, if the students decide and choose their research subjects after their arrival to the Zoo.

3.3 Materials

For this field observation students will need data sheets, pen and a stopwatch (contemporary cell phones usually provide a stopwatch function). It is also recommended that students bring a workbook, where they can record notes and other details that will help them to write the research report later. The official data collecting sheets can be printed out from the website of the Ethology Department, however if somebody forgets to do so, the demonstrator will provide these to the students before the practice.

4. CONDUCTING THE FIELD OBSERVATION AT THE ZOO

4.1 Goals

Each student should conduct individual observations, which means that everybody has his or her own research question, fills in his or her own data sheet and performs the data analysis individually. If the students work in pairs (which is allowed), this should involve reciprocal technical help only, but still, both students in the pair should have an own study. The technical help can be still very useful, as for example while one student watches the animals and handles the stopwatch, the other writes down the data to the sheet. Each student should perform **TWO** individual observations, by completing two sets of data sheets, obviously. A mandatory element of the practical is the submission of the (1) *field report sheet* and the (2) *practical report* for both observations.

4.2 Time range of the observations

The observations should last as long as the required amount of data is collected. The amount of data should be enough for reliable data analysis. As a general rule, 30 data points are usually sufficient for drawing conclusions from the results. Depending on the time interval chosen for the sampling, an observation can be as short as 15 min (30 s sampling), or as long as an hour.

4.3 Field report and data sheets

Students should complete the field report and data sheet while they are at the site of the observation. This, supported by a sketch of the observational area, provides the 'field' part of the report. In each case, the field report sheet must contain the following important data, without these the practical report cannot be evaluated.

- Name of the student
- Date and time (interval) of the observation
- Weather conditions (temperature, sunny/cloudy, rain yes/no, etc.)
- Subject(s) (scientific and common name of the species)
- General description of the group or individual animals (number, age, sex, other relevant features)
- Description of the observational area and circumstances (sketch is highly recommended)
- Research question (this must be a question that can be answered with yes or no)
- Selection criteria of the subjects, sampling method
- Features of the subject(s), how to identify them
- Results of the preliminary observation: this part contains the description of the behavioural elements that were used for the observation (do not forget the three parameters: posture, location, orientation)

The other part of the field report is the data sheet (see in the Appendix). This simple sheet provides rows and columns – the rows are usually serve for separating the time intervals step by step, while the columns can be used for various purposes, depending on how many subjects, behaviours, spatial divisions etc. we observe separately. According to the chosen method, we can put numbers (e. g. number of animals in a given area), abbreviations (e.g. of behavioural elements), or hash marks (e.g. frequency of behavioural element) to the individual cells of the sheet.

4.4 Written report

In this most important part of the student's work a short description should be written about the background of the observation, giving a reason for the research question (what kind of preliminary observation, or knowledge encouraged the student to choose that particular subject and question). It should be also mentioned, why and what kind of subjects were chosen, as well as why the given behavioural sampling method was selected. In the practical report the student should describe the special circumstances of the observation and the collected behavioural elements/ variables, too.

The main part of the practice report is the presentation of the results. Answering the research question is only possible if the results originate from the calculations, based on the collected data. A mandatory part of this section is the graphical illustration of the results, which is done by computer-generated graphs.

As the topic and the method of the observation can be highly variable during this practical, it would be hard to decide ahead, which type of statistical analysis will be suitable for processing the data. Therefore the students should ask the demonstrator after the observations were done, who will advise a proper way how to analyse the data.

After the results were presented, it should not be forgotten that one must discuss the results. In this closing chapter of the report the student should compare the results to the hypotheses, which were raised along the research question. It is useful if the student gives also an evaluation of the appropriateness of the chosen method retrospectively.

4.5 The evaluation of the report

The demonstrator will evaluate the students' work based on the following criteria:

- Field report sheet (original or scan) and written practical report (both are mandatory)
- Completed data collecting sheet
- Sketch of the observational area (whether it is in suitable size and including the important details)
- Research question (must be answerable with yes or no)
- Appropriateness of the chosen method of observation, list of the observed parameters or behavioral elements
- Were there enough data collected?
- Does the practical report cover the introduction, methods and hypotheses of the observation?
- Were the results calculated and analyzed correctly?
- Presence and quality of the illustrations for the results.
- How detailed and imaginative is the discussion? Did the student discuss the appropriateness of the chosen method, did he/she propose further investigation plans?
- Aesthetic appearance of the report.

Figure I.1 Field report sheet

Field report sheet

Name of the observer: ……..

Date of the observation: ……..

Weather conditions: ……..

Observed species: ……

Basic characteristics of the observed animal(s): (*quantity, age, sex, other relevant features*):

Description of the observed animals' environment (*a simple sketch is recommended*)

Research question (*it should be answerable with 'yes' or 'no'*)

Method of data collection:

How did you manage to track (recognize) the observed animal(s):

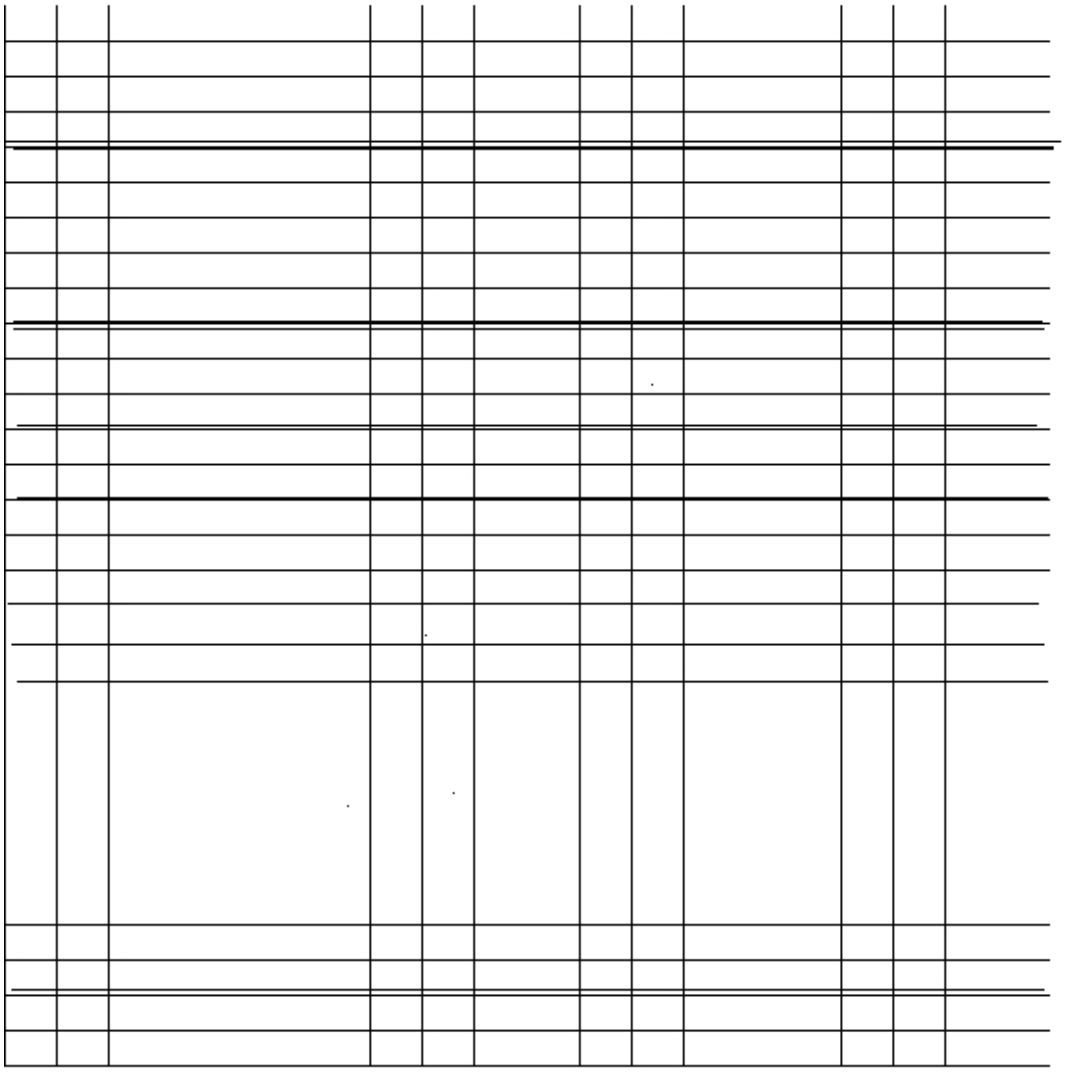
Description of the chosen behavioral units (*if there were any):*

Figure I.2. Field data collection sheet

Field data collection sheet

Name of the observer: ..... Date of the observation: .....

Observed species: .....



Codes of the behavioural elements (if there were any):

Characteristics of the observed animals (if there were any):

Notes:

5. LITERATURE CITED

Csányi Vilmos 1994. Etológia, Nemzeti Tankönyvkiadó, Magatartásvizsgáló módszerek pp.78-117.

Chapter 20 and 21 (this volume)

Chapter II. The ontogeny of antipredator behavior in fish fry

Péter Pongrácz

1. OBJECTIVES

In this chapter we discuss the various types of predator avoidance, including how experience modifies the inherited mechanisms of antipredator behaviour. We introduce some of the basic concepts of ethology, like the key stimulus and ontogeny, as well as the interactive model of learning. The practical includes experimental work on living fish fry. Students can test the effect of some factors that modify the inherited antipredator reaction elicited by the most important key stimuli. By modifying the location and number of eyespots painted on a model of a predatory fish, we will investigate whether the natural configuration (two, horizontally placed eyespots) has stronger effect eliciting predator avoidance than other alignments of the key stimulus.

2. INTRODUCTION

2.1 Antipredator behaviour

It would be hard to find an animal species, which is not facing the danger of being eaten by predators (at least at particular times of its ontogeny). Even the mighty African elephants are vulnerable when they are young and their size does not protect them from the largest of the carnivores yet. However, most animals are prone to threats of some kind of predator throughout their entire lifetime. It is not surprising therefore that there is a wide array of antipredator behaviours that were described in a multitude of species.

Antipredatory behaviours can be sorted in two main clusters. The so-called **primary defense mechanisms** aid in escaping the detection by a predator. These behaviours and the anatomical features that serve the primary defense can be called as **crypsis**. A few examples for the cryptic mechanisms are the **transparency**, **mimicry** and **changing of the colouration**. Once the animal was detected however by a predator, and the actual capture seems to be imminent, the only hope for to escape is the employment of one of the so-called **secondary defense mechanisms**. Among these we find various forms of discouraging, attention distracting tactics, as well as more direct threatening or combating of the predator. Just a few examples are the **self mutilating**, **feigning death**, **fighting back**, **threatening** and the **mobbing**.

2.2 Predator recognition

Avoiding the attack of a predator can be enhanced if an animal is capable of recognizing its enemy on the basis of some of the typical features of the predator. Among these auditory, chemical, visual, vibration cues can equally be found. Just like the other main behavioural categories of an animal, **predator avoidance is based on genetic and learned components** as well. How these two are interconnected can be understood with the help of the **interactive model of learning**, described by Csányi (1985, 1986). One of the main lessons of this model is that an animal does not necessarily escape/avoid immediately when it detected a predator (as one could expect it knowing how the key stimuli elicit unconditioned evasive reactions). Contrary, when an animal **detected a predator** (or more precisely: some of the key stimuli of a predator), without an imminent attack the animal will show rather **curiosity** and **exploration** instead of fleeing. Exploration serves a very important role: animals learn how to **differentiate a truly dangerous predator from a somewhat similar, but harmless creature**; or even how to recognize the telltale signs of a satiated (non-dangerous) or a **hungry, therefore dangerous predator**. As Csányi's model explains, learning additional information connected to particular key stimuli has an adaptive advantage for the animal, which will be able to decide to escape only when it is truly necessary.

2.3 Inherited recognition of predators

It was found in many species that they react automatically with **avoidance/ escape to particular key stimuli** without learning (in other words, without any previous unpleasant experience). In Scandinavia, where grazing deer present a danger for young pine plantations, odours of different carnivores were tested as deer-repellents (Sullivan et al., 1985). Interestingly, the results showed that not the sympatric (local) predators had the strongest repellent effect, but the extract of lion faeces deterred most effectively the deer from grazing on pine seedlings. As these deer were surely not exposed to lion attacks previously in Sweden, their **evasive reaction** to the smell of lion was most likely an **inherited** one.

There are many ethological studies that investigated the role of the **visible key stimuli** of predator avoidance. Maybe the most important of these is the horizontally positioned pair of eyespots that often elicit cautiousness or even fleeing from the potential prey animals. The adaptive value of this reaction is easy to understand: predators that hunt mainly based on their vision, usually have two large, ahead-looking eyes (these provide the proper 3D vision in front of the predator). If an animal is under the imminent threat of predatory attack, probably the most important sign of it is the sight of **two large, ahead-looking eyes** at the same time. This usually means that the predator has spotted its prey and by staring at it motionlessly, the final charge will follow soon. Interestingly, the proof of this mechanism comes not only from the investigations of the behavior of typical prey species (in mice: Topál et al., 1994; in paradise fish: Altbäcker & Csányi, 1990). The sight of two large eyes can surprise a predator itself – and this effect was favoured by evolution in many potential prey species, like some of the moths for example. When such an eyespot-bearing moth notices danger, suddenly exposes the eyespots hidden under its first pair of wings. Birds, like a blue jay show hesitation or with a startling response for such a display (Schlenoff, 1985), thus the moth is provided with vital seconds to escape.

The sight of the eyespots is regarded as an inherited key stimulus for predator avoidance. Its effect was extensively investigated with the help of a tropical fish species, the paradise fish (*Macropodus opercularis*) by the researchers of the Department of Ethology at the Eötvös Loránd University. It was found that not only the presence of the eyespots, but their number and their configuration are equally important for eliciting the proper evasive reaction. For example, one, three or four eyespots painted on a predator model were much less effective than two; and if two eyespots were painted in a vertical configuration they were not as effective as **two horizontally placed eyespots** (Csányi, 1986). Other features of a predator (colour, contour, size) had smaller importance compared to the role of the eyespots. When paradise fish were receiving painful stimuli (electric shocks) parallel with their exposure to a predator model, only when the model was equipped with the proper eyespots has the conditioning of the avoidance behaviour (model + pain → escape) happened effectively in the paradise fish.

2.4 Predator avoidance and the ontogeny

In most of the studies predator avoidance elicited by key stimuli was investigated in adult animals. It is logical from the aspect that the full-blown behavioural repertoire is usually present when an individual has reached its maturation. At the same time one can expect that behavioural forms that have not been modified by learning yet can be observed mostly in the young (or very young) animals. Predator avoidance is also very important even for the youngest of many species, because the risk of being eaten is especially high while the animal is young, weak and inexperienced. For example in wild rabbits it was found that before the rabbits would reach the 350 g body weight, the young generation loses 2-3 % of animals daily due to predator attacks in Australia (Richardson & Wood, 1982). Vitale (1989) conducted field experiments with simulated predator attacks on wild rabbits, and it was found that the young animals show less sophisticated avoidance behaviour and emerge sooner from the burrows after fled there from a predator, than the adult rabbits. Thus we can conclude that in rabbits the juvenile animals are not only easier to catch because they are weaker than the adults, but their survival is also hampered by their less-developed predator avoidance behaviour.

Fish in general offer useful experimental material for the investigation of the ontogeny of antipredator behaviour. Fish fry are small, develop quickly and in most species they are independent from their earliest age. All in all they are excellent subjects for comparing different age classes and examining how the antipredator behavior reaches step by step its mature form, or to investigate the specific ways of juvenile predator avoidance. In fish the transitional period between the larva and fry state is especially important, because when the fries start to swim (leaving behind the mostly bottom-laying larva state) they face an immediate and serious threat from predators. Working with the fries of the paradise fish, Hungarian ethologists discovered the formation of more and more sophisticated

antipredator behaviour as a result of the interaction of **ontogeny (gene-based development) and the environmental factors (learning)**. This complex process is often called as **epigenesis**. These experiments helped the scientists to identify many of the inherited key stimuli of predator recognition, as well as discovering some new learning phenomena.

Paradise fish fry start swim around in a greater extent when they reach the 10-15 day age. After hatching they are taken care of by their father, which collects and returns the accidentally scattered, hapless larvae to the so-called foam-nest, built by him on the water surface. We present here the results of a few experiments that were conducted on independently swimming and feeding fries of 15, 20 and 25 day of age. In each case the tests were done in small, elongated (20x5x5 cm) tanks. In one end of the tank the predator model was inserted, while the subjects were released one by one to the opposite end of the tank. From the several behavioural elements that were recorded, the 'retreat' and 'jumping' were especially important. Both served as moving away from the vicinity of the model. Additionally, the initial advancing of the fish to the model was characterized by the latencies of the individual entries to the compartments which were 1 cm wide sections of the tank, divided by lines painted on the bottom of the tank. Standard transparent laboratory ultracentrifuge tubes served as predator models. The tubes were filled with sand, and black eyespots were painted on their rounded ends (see Fig 1). Each subject was tested only once, and each test lasted for 3 min.

In our first experiment (Miklósi et al., 1995) we investigated the onset of the aversive effect of the horizontally placed two eyespots in different age groups of fries. We tested 15 and 20 day old fish with two-eyed and eyeless models. The results showed that paradise fish fries show avoidance behaviour only, when they were facing with the two-eyed model, while the eyeless model did not elicit antipredator response. However, the eyespots did not have any specific effect on the 15 day old fry. This experiment proved that the sight of eyespots becomes a key stimulus of predator avoidance between the age of 15 and 20 days in paradise fish fry.

In the second experiment we used only the 20 day old age group, and the role of the number and configuration of eyespots was tested. There were one-, two- and three-eyed predator models, and the two eyespots were presented either in a horizontal, or a vertical configuration. The fish showed significantly more intense predator avoidance in the presence of the model with the two, horizontally positioned eyespots than any of the other model variants. These results proved that the eyespots serve as key stimuli for predator recognition only if they are present on a predator-like object in their natural configuration (horizontal) and number (two).

Another study (Miklósi et al., 1997) was about to find out the answer for an interesting phenomenon: the reason why does the strong predator avoidance reaction of the 20 day old fish disappear if we test 25 day old fry with the most effective model type (equipped with the two, horizontally placed eyespots). It was found earlier that the 25 day old fish does not show antipredator behavior when they were tested with the above mentioned model. The role of ontogeny seemed to be unlikely as (1) the adult paradise fish react with avoidance to the sight of the eyespots as well, and (2) the 25 day old fry are just as threatened by predators as the 20 day old age class. Therefore we tried to modify the environmental effects that may have affected the development of predator avoidance between 20 and 25 day of age. Half of the subjects were raised in the usual way, where they were kept in groups of 30-40 fish in small, 6 l aquaria. The other half of the subjects received 1, 3 or 7 days long isolation before they reached the 25 day old age. These fish were separated from their shoalmates, and they were housed individually in 6 l aquaria for the given length of time. The tests were conducted in each case when the fish were 25 day old. Two-eyed and eyeless models were used as predator stimuli. The results showed that while one day of isolation was not long enough to affect the behaviour of the fry, they showed similar predator avoidance after three of seven days of isolation, than the 20 day old fry. Importantly, only the two-eyed model elicited antipredator responses. This experiment showed that the effect of key stimuli can be overwritten by learning (**habituation**[1]), if the fries live in high density. In such an environment they are constantly exposed to the sight of their shoalmates' eyes. However, the effect of habituation is reversible, and it disappears after a few days of isolation (or low-density living environment). In the nature, 20-25 days old fries have already been scattered among the water plants, therefore they do not have opportunity for being habituated to the sight of the eyespots of other fish.

3. MATERIALS

3.1 Test subjects

During the practice 5-10 days old fries of the guppy (*Poecilia reticulate*) are used as test subjects. Each fish is tested only once. Guppies are bred and raised at the Department of Ethology. Fries are kept isolated from each other for three days preceding the tests.

3.2 Experimental device

The testing tanks are small, elongated aquaria, with dimensions of 20x5x5 cm. The walls of the tank are painted mid-green from the inside. The floor of each tank is divided to 1 cm wide cross-sections, which are marked with black lines. One end of the tank serves as the starting compartment for the subject, while the predator model can be inserted to the opposite end of the tank. Before the next subject is released to the start compartment, the tank is re-filled each time with fresh water of 26 Celsius degrees of temperature. The water should be 3 cm deep in the tank. A small net is used for lifting the subjects from their keeping tank to the test tank, and after the test the fries are returned to their own tank again with the same net. As the walls of the test tank are painted opaque, the subjects can be observed during the test from above, with the help of a mirror, which is positioned at 45 degrees of angle over the tank.

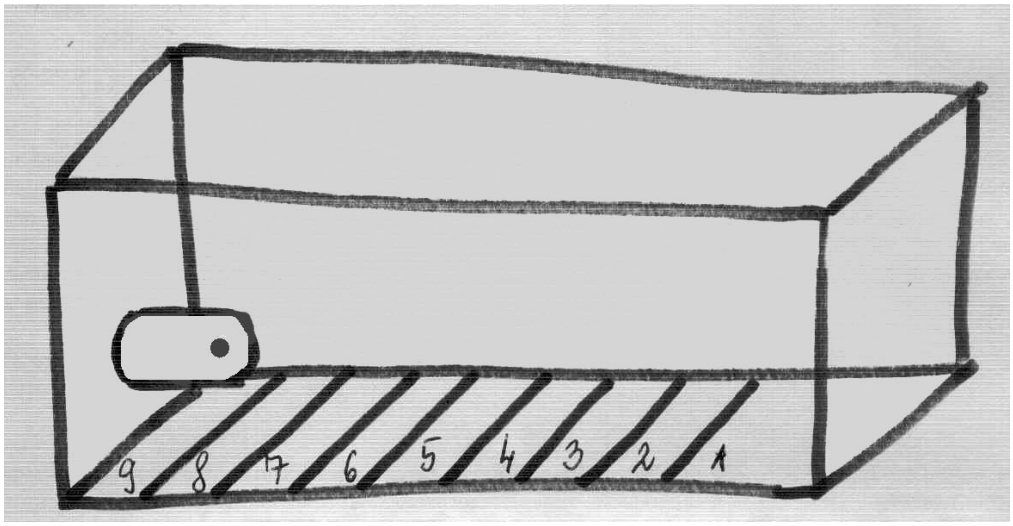


Figure II.1: Test tank for fish fry. The small tank is 20 cm long, 5 cm tall and 5 cm wide. On its left end a predator model is attached to its wall. Each subject is released at the opposite end, in the start compartment ('Compartment 1'). The lines drawn on the floor of the tank separate the cross-sections that are used for describing the subject's advancing against the model.

4. PROCEDURE

4.1 Goal of the practical

The question of the experiment is whether fish fry react differently to models of predators depending on the amount and configuration of the eyespots painted on the predator. We follow the methodology used by Miklósi and colleagues (1995), but here we use guppies instead of paradise fish as subjects. According to our hypothesis, just like the paradise fish, guppies will show the strongest predator avoidance in the case of the two-eyed predator model, on which the eyespots are painted in a horizontal configuration.

4.2 Experimental process

Each fish is tested for three minutes. The test starts when the fish crosses the line between compartments 1 and 2.

Before the release of the next subject, the corresponding predator model should be inserted and fresh water should be poured 3 cm deep to the tank. When the tank is positioned properly under the mirror, using the small net carefully and gently, a fish is released to the start compartment. It is important that fries should not be dropped to the tank from the air, but be released by submerging the net to the water. We should let the fry slip from the net right to the water. When the subject entered the starting compartment, we remove the net slowly and carefully, and wait for the fish starting to move. When the fry crosses over the line between the 1st and 2nd compartment, we start measuring the three min long trial. If the fish does not leave the start compartment for three min, we make a note of it and exclude the subject from the test. After returning it to its keeping tank, we switch the water in the test tank, and continue the procedure with a new subject.

Students work in pairs during the behavioral observation. A recommended sharing of the tasks may be that one of the students watches the fish in the tank and tells what happens, while the other member of the team writes the behavioural elements to the data collecting sheet and handles the stopwatch. The following parameters should be collected:

- number of compartment switches (how many times did the fish swim over the lines that separate the compartments)
- **latency** (s) of entering compartment 8 (this is the time elapsed until the fish swims over first time the line between compartments 7 and 8. If the subject does not enter compartment 8 at all, this latency is 180 s)
- number of retreats (the fish stops, then slowly moves backward, while its body typically forms a slightly curved hook shape)
- number of jumps (this is a sudden, fast leap against the preceding direction of the locomotion. Fish may jump after it stopped, or was just retreating, but jumps can occur right in the middle of a swimming forward, too. Fish jump almost always to the opposite direction than they were facing at before)

When the three minutes were elapsed, the test is over, and the subject is returned to its own tank. Each pair of students tests one subject with each predator model.

4.3 Experimental groups

The following predator models will be used (each of them is 1 cm of diameter):

- eyeless model
- model with two, horizontally positioned eyespots
- model with two, vertically positioned eyespots
- model with three, horizontally positioned eyespots

4.4 Data analysis and the presentation of the results

At the end of the practical the pairs of students prepare such summarized data sheets, which contain the columns of all the data collected in the same test conditions. For example, the number of compartment switches, latency, number of retreats and jumps of each fish tested with the eyeless model will be sorted to separate columns. During the data analysis we will compare the parameters of the different experimental groups. We expect Gaussian distribution for most of the parameters, however, this should be tested at first with Kolmogorov-Smirnoff test. In the case of Gaussian distribution we will perform one-way ANOVA with Bonferroni post hoc test. In a case of non-Gaussian data distribution we will use non-parametric Kruskal-Wallis test with Dunn's post hoc test.

Each member of the student-pairs performs the data analysis and writes the practice report individually – in other words the co-operation is restricted to the data collection phase only. The practical report should present the results according to the following guidelines:

- The raw data of the four fish that the pair tested should be presented in a table format.
- In the case of each parameter a graph should be created that shows the results of the four experimental groups. Do not miss to indicate the significantly differing groups (if the statistical analysis found significant effect)[1].

[1]See Chapter 20 (Statistical analysis)

- Results of the statistical analyses should be presented in a table format (even if the difference was not significant).

4.5 Preparing a report

The report is a mandatory part of the experimental work. Each report should contain the following parts beside the above mentioned presentation of the raw data, statistical analyses and results:

- Introduction – where the author reviews the theoretical background, aims, research question and hypotheses of the experiment.
- Materials and methods – the author provides a clear description of the subjects, equipment and procedure of the experiment.
- Results – statistical analyses, graphs, and the table of the raw data collected by the author and his/her team partner.
- Discussion – the author compares his/her results to the findings of similar researches. The author discusses the results in the light of the experimental hypotheses. It is useful if the author tries to find broader conclusions of the actual experiment.

4.6 Evaluation of the report

While evaluating a student's work, the following details are examined:

- Did the student write a detailed introduction, including the scientific background of the research, the experimental question and hypotheses?
- Did the student explain the methods and materials of the experiment?
- Were the necessary statistical analyses performed and presented in the report?
- Were the results illustrated with acceptable graphs/ figures?
- Did the student explain and discuss the details of the results?
- Were the mathematical formulas and statistical analyses correct?
- Does the report include a general discussion, where the student draws the broader conclusions of the study, and connects the new results to the former knowledge based on the literature?
- Does the report fit to the formal and aesthetical requirements?

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Chapter III. Search image formation in domestic chicken

Gabriella Lakatos

1. OBJECTIVES

The goal of the present practical is to exercise the rules of experimental work with live subjects, and to observe the behaviour of free-moving animals and describe their behaviour (e.g. developing and using an ethogram). Further goal of this lesson is to examine the search image formation in chicks according to a predefined experimental protocol and to get experienced in statistical data analysis.

2. INTRODUCTION

The search image hypothesis was originally proposed to account for the observation that animals selecting among different kinds of food often consume an excess of the more common type. The hypothesis states that animals searching for a particular cryptic food item focus on visual features that are characteristic of that item, thereby facilitating its discrimination from the background (Tinbergen, 1960; see also Bond and Riley, 1991). Hereby, they form a search image for the certain grain type.

Alexandra Pietrewicz and Alan Kamil (1979) investigated the search image formation on blue jays (*Cyanocitta cristata*). Birds trained to detect Catocala moths in slides were exposed to two types of slide series containing images of these moths: series of showing only one of the two species and a series showing the two species intermixed. In one species series, detection ability increased with successive encounters with one grain type. No similar effect occurred in two species series. These results are a direct demonstration of a specific search image.

Bond and Kamil also examined the question of search image formation in blue jays. Their results showed also that detection performance was strongly facilitated in the course of a sequential priming but was relatively unaffected by sequences of mixed target types. Detection accuracy in subsequent probe trials was enhanced by priming with targets of the same type, whereas accuracy on cryptic probes following a priming with a more conspicuous target was significantly degraded. Their results hereby support the ‘enhanced attention’ hypothesis instead of the searching image hypothesis for the high predation ratio on the more abundant prey.

In a further experimental study, conducted by Plaisted and Mackintosh (1995), the detection of cryptic ‘prey’ was examined in pigeons (*Columba livia*) using an operant discrimination procedure and complex computer-generated stimuli. In their experiments they manipulated the frequency with which each of two target types appeared, and they found further evidence for Tinbergen’s claims that a high-frequency target is better detected than a low frequency target. Their results also suggested that an uninterrupted ‘run’ of encounters with one cryptic target facilitates performance and that this facilitation does not appear when two targets appear intermixed.. Since the two targets in the study were equally cryptic, results of these experiments provide evidence consistent with the search image hypothesis.

Studies with blackbird (Lawrence, 1985) provided similar results, supporting the hypothesis that the formation of search image for a given grain type enhances the efficiency of prey detection.

Similar studies were also carried out on chicks (Dawkins, 1971) using different coloured grains, which were presented on a different coloured background for the birds. These studies demonstrated that, although the chicks were initially unable to detect the coloured grains of rice dyed the same colour as the background was, subsequently a significant improvement in performance was observed in the chicks’ food detection. This change is most plausibly seen as a central change of perception. Ability to see cryptic rice was not fully retained from one day to the next. On the other hand, feeding chicks on conspicuous grains had an adverse effect on their ability to detect cryptic grains. These results are in line with L. Tinbergen's hypothesis that birds may use 'searching images'.

Further research (Dawkins, 1971b) have also shown that the chicks are able to shift their attention quickly between the conspicuously coloured and the cryptic food, depending on what kind of food they are eating at the time.

3. PROCEDURE

1. Group discussion of theoretical background (see the Introduction) of the tests, the presentation of the experimental equipment, explanation of the protocols.

2. Explanation of the Data Collection Sheets.

3. Conducting the experiments. The chicken should be given 20 minutes rest between each test. We will share the experimental data in the group and perform the statistical analysis on the complete data set.

4. Discussion of the results.

3.1. TEST 1: DETECTION OF CRYPTIC PREY

3.1.1. *Hypotheses and predictions*

Prior to the test, over seven days the chicks were fed on a certain colour food. The aim of this specific test was to study whether the chicks form search image for this type of food and whether they are able to detect it on a same coloured background.

The two main questions of this test are:

1. Whether the chickens' cryptic food detection performance is getting better with the time?

2. Whether the detection performance of chicken is better if the grain type is conspicuous against the background compared to when it is cryptic on the background?

Based on the literature described above, we have the following predictions:

1. We assume that the chicks will find the cryptic coloured food with a growing rate in time, which suggests that each chick forms a search image for this particular type of food on the basis of its' visual characteristics.

2. We assume that the chicks will find in a higher proportion the conspicuous food than the cryptic food.

3.1.2 Behavioural analysis – Data collection

Experimental protocol

Half of the chicks were fed by original coloured (yellow) grains for seven days prior to the experiment, while the other half of the chicks were fed by green coloured grains.

In the first test, we examine the chicks' food detection performance if they meet the previously trained grain type on a same coloured background. We also examine whether their performance increases by time.

To study these questions we will present the food to the chicks on two different coloured background, same colour background (the food will be cryptic), white background (the food will be conspicuous). Half of the chicks will be tested with the same colour background for the first time, while the other half of the chicks will be tested with the white background. We have to have at least 10 minutes break between the two subtests.

The performance of the chicks will be measured by analysing the chicks' pecking behaviour (frequency of pecking). We will measure fifty pecking in both subtests and in each case we will record the latency of the pecking behaviour (that is the time elapsed from the start of observation until the pecking was detected) and the total length of the subtests. At the end of the test we will calculate the sum of the duration for the first five and the last five pecking.

3.1.3. Coding sheet:

We will record the chicks' behaviour on the following coding sheet.

DATA SHEET FOR SEARCH IMAGE FORMATION			DATE		
			EXPERIMENTER		
Peck	**Latency**	**Duration (For the first and the last five peckings)**			
1			26		
2			27		
3			28		
4			29		
5			30		
6			31		
7			32		
8			33		
9			34		
10			35		
11			36		
12			37		
13			38		
14			39		
15			40		
16			41		
17			42		
18			43		
19			44		
20			45		
21			46		
22			47		
23			48		
24			49		
25			50		
Duration of the test:			**Duration of the test:**		

3.1.4. Data analysis

For the statistical analysis we merge the data of all the chicks.

For analyzing the chicks' performance in case of the differently coloured backgrounds we use Wilcoxon match paired test. We will compare the pecking latencies in case of the two different kinds of background, as well as the durations of the first five and the last five pecking.

For the statistical analysis we use the software „INSTAT", following the recommendations of Chapter 20-21.

3.2. TEST 2: Formation of search image when multiple grain types are available

3.2.1 Hypotheses and predictions

Questions for the second test:

1. Will the chicks consume the previously trained grain type in a higher proportion when there are two different grain types available in parallel at equal abundance, and the two grain types are equally conspicuous on the background?

2. Do any changes occur in the chicks' performance of finding the previously trained cryptic food (on a same colour background) following a session when the two different food types were presented simultaneously?

Based on the literature described above we have the following predictions:

1. We assume that if the chicks form a search image for the previously trained grain type, they will consume more from this kind of food. It is also possible that in case of the presence of two, equally abundant grain type they do not use search image, in this case there will be no difference in the pecking frequency on the two grain types.

2. We assume that the chicks' performance of finding the cryptic food will decrease after a session when the two grain types were presented for them at the same time.

3.2.2 Behaviour analysis – Data collection

Experimental protocol

The experiment is carried out exactly as the first test was, with the difference that in this case two different types of food were presented for the chicks first, on a white background (paper sheet), scattered in equal abundance. Subsequently, as in the previous experiment, we will present the previously trained food type on a same colour background (the food will be cryptic).The pecking behaviour will be coded. For both subtests fifty pecking will be measured. In case of the first subtest, pecking frequency of the two food types will be recorded. In addition, we will record the latency of the pecking behaviour (that is the time elapsed from the start of observation) and the total length of the subtests. At the end of the test we will calculate the sum of the duration for the first five and the last five pecking.

3.2.3. Coding sheet

Please, mark with an X on the sheet in case of each pecking whether the chick pecked the previously trained or the other type of food.

DATA SHEET FOR SEARCH IMAGE FORMATION				DATE			
	TEST 2			EXPERIMENTER			
Peck	**Previously trained colour**	**Non-trained colour**	**Latency**	**Peck**	**Previously trained colour**	**Non-trained colour**	**Latency**
1				26			
2				27			
3				28			
4				29			
5				30			
6				31			
7				32			
8				33			
9				34			
10				35			
11				36			
12				37			
13				38			
14				39			
15				40			
16				41			
17				42			
18				43			
19				44			
20				45			
21				46			
22				47			
23				48			
24				49			
25				50			
Sum:			Sum:	**Sum:**			Sum:

For the second subtest we will use the same coding sheet, which we used in the first test.

3.2.4 Data analysis

For the statistical analysis we merge the data of all the chicks. For the comparison of the pecking frequencies in the case of the two different prey-types we will use Wilcoxon matched pair test. We use the same kind of test for comparing the pecking latencies in the case of the two prey-types.

To answer our second question we compare the chicks' performance in the first test (when using cryptic food) and in the second subtest of the second test.

For the statistical analysis we use the software „INSTAT".

3.3 Preparation of the report

Each student need to write a separate work report!

The report shall include:

- A brief introduction
- Questions
- Hypotheses, predictions
- A brief description of the method
- The results obtained and their short assessment.

3.3.1 Discussion

Answer the questions of the two tests according to the following points

1. Describe the differences you have found during the statistical analysis.

2. Please, explain whether these differences/similarities confirm or refute the basic hypothesis.

3. What do the results say about the search image formation?

4. Do you have any other idea on the basis of the introduction for how to examine search image formation on birds?

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Chapter IV. Operant conditioning in the practice

Márta Gácsi

1. OBJECTIVES

The practical is designed to provide students insights into one of the classic and still widely used methods of behaviour studies, and give them a chance to try the method in practice. They will get acquainted with the ethological approach and behavioural interpretation of learning, the basic forms of learning theory, and a concrete aspect of its application. During the practical live dogs are present as test subjects. In addition, students have the opportunity to condition simple tasks on their own. First, they can practice on each other and try out the main steps of operant conditioning in order to experience personally the essence of the method from the subject's point of view. Second, they condition the dog to perform a simple task, then, in the case of a more complex shaping (done by an experienced trainer), they code and analyse the observed behaviour.

2. INTRODUCTION

2.1 Theoretical Overview

Examination of the learning abilities of animals has always been of particular interest in behaviour research. Emphasizing the importance of controlled and accurately reproducible experiments performed in laboratory environment, behaviourists mainly focused on the detection of general learning mechanisms looking for answers for ultimate questions of human behaviour and learning.

According to the ethological approach, it is essential that – like other forms of behaviour – the learning abilities of a species have also genetic components. Consequently, it is selective and closely linked to inherited forms of behaviour, that is, not any arbitrary association can be taught to animals even those with advanced learning capabilities.

Depending on the focus of examination, *learning* can have a wide variety of definitions, but most generally it can be defined as a biological process by which the behaviour of the individual changes in the long run due to some kind of environmental impact or experience.

The genetic information is fine-tuned by neural learning, which helps the adjustment to temporary or less predictable impacts. Innate behavioural traits (genetic memory) and behavioural responses developing because of learning from environmental impacts (neural memory) always interact closely with each other.

2.2 General forms of learning

Two of the most common forms of learning, typical even for species with relatively simple nervous system, are the process of **habituation** and **sensitization**. These forms of learning occur in the case of repeated stimulation and have opposite effects on the responsiveness. Habituation occurs when repeated presentations of the stimulus cause a decrease in the response because the animal gets used to the stimulus. The likelihood of habituation is dependent on the nature of the stimulus, the rate of stimulus presentation, and the regularity with which it is presented. During sensitisation there is an increase in a response after repeated presentations of the stimulus. The stimulus has to be important (intrinsically unpleasant or aversive) or unusually strong. Therefore, the same stimulus can be neutral for a species and very important for another.

There are specific learning processes observed during the ontogenesis of precocial birds and some mammals, playing a role mostly in conspecific recognition. These processes take place during a relatively well-defined early period of development and they are characterized by rapid and hardly reversible learning. This special kind of early learning is typical, for example, when the parent’s characteristics are “imprinted” into the nervous system

of the offspring. The modern interpretation of the phenomenon of **imprinting** (e.g., Bateson 1981), however, does not formulate as strictly as the original theory, and talks about "sensitive" rather than "critical" periods, which refers to more flexible learning and less clearly irreversible effects.

Although contemporary neurophysiologists addressed and revealed a number of crucial aspects of learning processes, the actual behaviour of the animals was mostly attributed to intrinsic responses or conditional responses resulting from simple **associative learning** effects. Various forms of associative learning have been studied on many species in laboratory experiments, in which the animals had to recognize the connection between two events occurring close together in time.

Pavlov's famous experiments on dogs (1927) showed that the recognition of the relationship between two events (a bell's ring and the appearance of food in the original experiment) can be demonstrated by behavioural changes. The response (the dog's salivation), which was originally showed only in the presence of the unconditional stimulus (food), could be triggered also by the conditional stimulus (bell) – that is, the originally neutral stimulus and the response were associated due to the reinforcement (food). Thus the unconditional response is a reaction to the biologically natural stimulus; the conditional response is a learned reaction to a signal. This form of learning is called **classical or Pavlovian conditioning.** Of course, classical conditioning works in case of not only training or laboratory conditions. It is a typical form of learning in animals in their natural habitat, which occurs when individuals recognize the connection between two environmental stimuli (an unconditional and a neutral one), and this is reflected later in their behaviour.

The second type of associative learning is **operant conditioning,** during which the subject recognizes the relationship between its own "spontaneous" behaviour and the subsequent motivating stimulus (consequence).

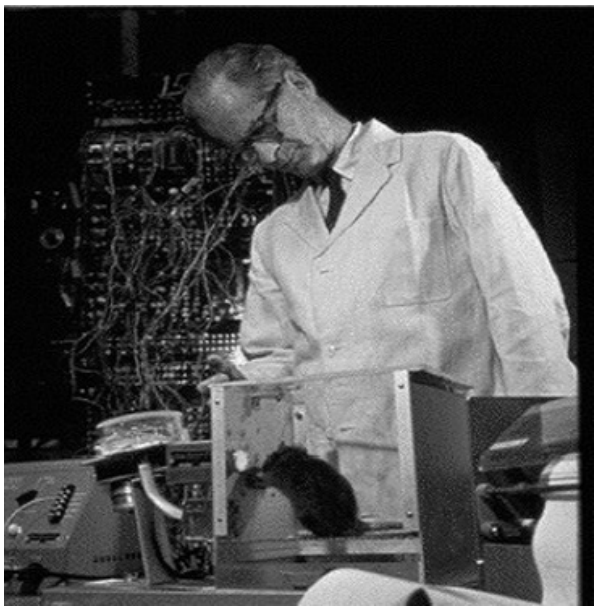

Fig 4.1 Skinner with his box in operation

The best known representative of early experimental psychology, L. Thorndike (1874-1949) introduced a small instrument as the classical device for comparative tests, called the "problem box". The box was in fact a cage, and the animal placed inside had to find its way out using "trial and error" learning. For example, the subject could obtain the food placed outside the box by manipulating the relatively simple locking mechanism of the box. According to Thorndike's **law of effect**, all behaviours have consequences and an important feature of all behaviours that the consequences have an impact on them.

The key concept of operant conditioning is **feedback.** Due to the consequences of the behaviour the probability of its reoccurring changes, that is, the frequency (or the probability of the occurrence) of a specific behavioural response increases or decreases through learning. Skinner, who developed the operant conditioning theory in details (Skinner1938), applied an experimental box operating under similar principles. The subject could obtain food by pressing a pedal, so i) the successful action has to be invented by trial-and-error learning, and ii) the animal was supposed to recognise the relationship between the action and the reward.

However, if we want to teach a complex or a very specific behaviour (for example, double somersault for dolphins) we cannot rely on trial-and-error learning, as there is no chance that the individual implements the specific series of movements or action on its own, because it does not know that it would be rewarded. Instead, we can apply **shaping**, when during the conditioning the required action is achieved by a gradual and continuous forming of the behaviour. The key element of this training method is that we gradually impose more stringent requirements and the subject will be rewarded only if it is getting somewhat closer to the final action, shows a little more approximate behaviour (for example, initially the dolphin is rewarded even when it jumps out of the water, etc.).

2.3 Operant conditioning as a training method: clicker training

The methodology of the clicker training comes from operant conditioning research conducted on animals (mainly on rats and pigeons) in order to gain better understanding of human learning. It quickly spread to animal training for various purposes.

The clicker training applies both forms of associative learning.

1. During the first training sessions an association between the clicker's sound and the food reward is to be established (classical or Pavlovian conditioning). The clicker itself is a small metal device that produces a short, distinct sound when pressed. Once associated with some primary reinforcer (food), the auditory stimulus (click sound) is used to provide immediate reinforcement for a correct response. Thus the clicker serves as a "secondary" or conditioned reinforcer that pinpoints a specific correct behavioural response even if the primary reinforcement (food) cannot be delivered at that precise moment in time. By using a clicker to bridge the delay between the expected response and the delivery of the primary reinforcer we can more accurately indicate the correct behaviour element's appearance even from a distance.

2. When the animal has already learnt the association between the click sound and the food, operant conditioning can be performed through gradual shaping of the behaviour. We can use the clicker (followed by food reward) to reinforce offered behaviours, which are close enough to the final, desired behaviour. With this simple method, the gradual formation of complex behaviours and longer behavioural sequences can be taught to the animal. The major advantage of this technique in dog training is that it is based solely on positive reinforcement.

The method originally developed to conduct psychological research quickly spread to a variety of uses in case of different animals as a training method. The core of the conditioning technique was first applied in dolphin training (using a whistle) in the 80s, which was adapted by dog trainers who searched for training methods based on positive reinforcement (Pryor, 1999).

Since then, the clicker training has found its way back to scientific studies, for example, it proved to be successful in testing some characteristics of social learning in dogs (McKynley, 2004). In the last decade, it has been widely applied in the behavioural study of other domesticated species (Ferguson & Rosales-Ruiz, 2001; McCall & Burgin, 2002;. Williams et al 2004) and it was successfully used in handling and studying of captive wild animals (Zulch & Harman, 2004). These results are also important with respect to animal welfare, because their application for practical purposes provides a non-invasive method for the handling of zoo animals (e.g., in the transfer of big cats when moving them from their cage to carry crates), or laboratory primates, who can be trained to cooperate with the veterinarian voluntarily on this way.

Finally we want to note that the conclusions of the experimental psychological approach of learning are somewhat weakened by the neglect of some important biological aspects: as the relevant **ecological environment** (species specific natural environment), the individual's own **past experiences**, the effects of social learning and the complex socio-cognitive abilities were ignored. Therefore, we need to keep in mind that in practice the "rules" of conditioning are not applicable automatically.

3. MATERIALS AND METHODS

A demonstrator who is experienced in the shaping of more complex actions using a clicker leads the practical. The protocol is valid for groups of 20-30 students.

3.1. Experimental animals and equipment

During the tests, two experienced family dogs' responses are to be observed in different learning situations. The dogs need sufficient experience in learning by clicker training and performing tasks in the presence of several strangers (e.g. therapy dogs).

Two clickers are needed, one stopwatch for each two students, and food reward for the dogs (adjusted to the dogs' size).

The data collection is conducted using paper data sheets, and then the data is transferred to Excel and analysed by using INSTAT.

3.2. Procedure

After a short theoretical introduction, the practical consists of three separate sub-tasks. The first merely serves to provide students with the minimum routine to use the method. At the second (conditioning of a dog for a simple action) and third (shaping) task, we determine the hypotheses, the method of measurement and data processing in advance, and the results are evaluated jointly.

The practical work is carried out in two approximately similar groups.

4. DATA COLLECTION

4.1. Practicing the method on mates – shaping the behaviour

One student leaves the room, while the rest of us invent a moderately complicated and not obvious behavioural sequence, which will be implemented (e.g. go to the black board take the sponge and put it into the waste bin). Two other students (in parallel) handle the clickers trying to shape the subject's behaviour; all actions deemed as appropriate are signalled by clicking, thus the student's responses are gradually shaped toward the specific goal. The rest of the students can interactively participate evaluating the clicker usage, but only without betraying the task to the subject. We apply two persons to click so that it is less likely that a single inexperienced 'trainer' would permanently lead the subject astray.

The aim of this warm up task is to quickly master the technique and understand the essence of the shaping. This aim is served by the parallel clicks and their continuous assessment by the students and the demonstrator.

In this case the reward is only "virtual", but the student has to touch the shoulder of one clicker person after every click, so that similarly to the dog, she/he should start the behaviour sequence from the beginning after every reward.

The task should be repeated several times involving new volunteers. (about 20 minutes)

4.2 Operant conditioning with dogs

The students are working in two groups simultaneously, in separate rooms and with different dogs.

Method

After the presentation of the task, we determine the methods to be used together.

Joint discussion of the following issues:

- What are the advantages and disadvantages of the possible measuring methods?
- What (type of) variables can we code?

The practice of the measuring procedure and the role of measuring pairs are necessary.

There is a need to evaluate the motivational value of the treat planned to be used. For example, we place a bit of treat 1-2 meters apart from the dog and if it immediately runs to consume it then we can start the task.

4.2.1. Test A – capturing a single simple action

Encourage the dog to establish eye contact with one particular student

One student holds the clicker in one hand behind his/her back so that the dog could not see it. The plate containing tiny treats is on the table about 2 m from him/her, next to the demonstrator who will drop the treat to the dog when the click sound can be heard. We define in advance the person with whom the dog need to establish eye contact. During the test the dog is free to move around and if it looks at the appointed person's eyes, the student is supposed to click and the demonstrator provides the reward. All participants have to follow the dog with their gaze and can't look at the appointed person during the test. Should the dog move away for a longer period of time and not orient towards the students, the demonstrator can lure it back by touching/knocking the plate to increase the motivation of the dog. (The eye contact is always interrupted when the reward is provided.)

Formulating the hypotheses and predictions

Based on the literature, what initial hypotheses can be formulated for the effectiveness of the conditioning?

What specific predictions are allowed by the selected variables?

For example:

- Due to the positive reinforcement, the frequency (probability of the occurrence) of the desired behaviour unit (eye contact) will increase during the test.
- The latency of eye-contact will decrease in the course of observation, thus the dog will gaze at the right person with decreasing intervals.
- Alternative hypothesis: There is no change in the frequency/latency of eye contact during the test…

Behavioural analysis – Data collection

Those participants who do not take part in the training, measure the latency of eye contact. (The students who handle the clicker and the food will use the data collected by the others.)

The data collection is done in pairs, using stopwatches and the attached notebook form.

One member of the pair measures the time that elapses until each click and tells it to the partner who registers it on the form. The stopwatch is to be restarted after each food reward, so that the subsequent latency data will be under each other on the form.

Data analysis

The calculation is done alone, not in pairs.

- Calculation of the mean latencies observed in the first and second part of the test.
- Combining of the calculated mean values with the data of the previously observed dogs (the previous results are available in an Excel file on the classroom computers).
- Performing the statistics using InStat program: normality test, group means calculations, paired t test. (The demonstrator actively assists in carrying out the statistical calculations.)
- Noting the calculated values: means, standard deviations, test statistic value, degrees of freedom, and significance level records.

Joint discussion of the results.

4.2.2 Test B - Shaping

A An experienced clicker user (demonstrator) performs the training. She tries to teach a new, moderately difficult task to the dog.

The task is always different: reversing, spinning, object nosing, opening fetch and carry objects, etc.

Formulating of hypotheses and predictions

Based on the literature, what initial hypotheses can be formulated for the effectiveness of the conditioning?

What specific predictions are allowed by the selected variables?

For example (are they testable?):

- Due to the positive reinforcement the frequency (probability of the occurrence) of a behaviour action that is closer to the desired behaviour will increase during the test.
- The latency of clicks will decrease in the course of observation, thus the dog will perform expected behaviours with decreasing intervals.
- There is no change in the frequency/latency of clicks during the test.

Behavioural analysis – Data collection

The data collection is done in pairs, using stopwatches and the attached notebook form.

One member of the pair measures the time that elapses until each click and tells it to the partner who registers it in the form. The stopwatch is to be restarted after each food reward, so that the subsequent latency data will be under each other on the form.

Data analysis

The calculation is done alone, not in pairs.

As the data of the two tested dogs alone are not suitable for statistical analysis, for further evaluation the mean values of the coded data will be added to an existing larger database. This way - due to differences in the coding - each group will have a somewhat different dataset and results of the statistical analysis.

The steps of data analysis are:

1. Calculation of the mean latencies observed in the first and second part of the test.

2. Adding the calculated mean values to the previously observed dogs' data (the previous results are available in an Excel file on the classroom computers).

3. Performing the statistics using INSTAT program: normality test, group means calculations, paired t test. (The demonstrator actively assists in carrying out the statistical calculations.)

4. Noting the calculated values: means, standard deviations, test statistic value, degrees of freedom, and significance level records.

Joint discussion of the results.

4.3. Preparation of a report

The report shall include:

- question
- hypotheses, predictions,
- brief description of the method,
- the results obtained
- and their short assessment.

Each student has to prepare a separate report!

4.4. General evaluation – Considerations for the discussion

- Have the collected data supported the hypothesis? Has any prediction been proved?
- Can the results be explained by any alternative hypothesis?
- Was the selection of variables relevant?
- What would you do differently if you had to re-do this test?

Answering questions together.

Figure IV.2. DATASHEET – measuring latency

Name:.....Pair: .....

Date: .....Group:.....Dog: .....

	Eyecontact – A latency		Shaping – B latency	
	part 1	part 2	part 1	part 2
Mean Latency				

Figure IV.3 Report Test A – Eye contact

Test A – Eye contact

Name: ……Date: ……Group: ……

Question:……

……

Hypotheses and predictions:

1. ……

……

2. ……

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Method:……

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Results

Part 1 Mean: ..... Standard Deviation .....

Part 2 Mean: ..... Standard Deviation .....

Stat. test: .....

Discussion:……

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Figure IV.4 Report Test B Shaping

Test B – Shaping

Name: ..... Date: ..... Group: .....

Question:.....

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Hypotheses and predictions:

1. .....

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2. .....

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Method:.....

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Results

Part 1 Mean: ..... Standard Deviation .....

Part 2 Mean: ..... Standard Deviation .....

Stat. test .....

Discussion:.....

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Chapter V. The effect of imprinting on the behaviour of domestic chicken

Gabriella Lakatos

1. OBJECTIVES

The goal of the present practical is to provide the students experience with the experimental work with small birds, and to observe the behaviour of free-moving animals and describe their early behavioural development. Further goal of this practical is to examine the phenomenon of imprinting in chicks according to a predefined experimental protocol.

2. INTRODUCTION

Imprinting is the term used in psychology and ethology to describe any kind of phase-sensitive learning (learning occurring at a particular age or a particular life stage) that is rapid and apparently independent of the consequences of behavior. It was first used to describe situations in which an animal or person learns specific characteristics of some stimuli, which is therefore said to be "imprinted" into the subject.

It is assumed that imprinting has a sensitive (critical) period. In general, the sensitive period is a limited time-window in which an outside event results in a specific developmental transformation. A "critical period" in developmental psychology and developmental biology is a well-defined time interval in the early stages of an organism's life during which it displays a heightened sensitivity to certain environmental stimuli, and develops in particular ways due to experiences at this time. If the organism does not receive the appropriate stimulus during this "critical period", it may be difficult, ultimately less successful, or even impossible, to develop some functions later in life.

2.1 Filial imprinting

The best known form of imprinting is the *filial imprinting*, in which a young animal acquires several of its behavioral characteristics about its parent. It is most obvious in nidifugous birds that imprint on their parents and then follow them around. It was first reported in domestic chickens, during the 19th-century. The phenomenon was rediscovered by the early ethologist Oskar Heinroth, and studied extensively and popularized by Konrad Lorenz working with greylag geese. Lorenz demonstrated how incubator-hatched geese would imprint on the first suitable moving stimulus they saw shortly after hatching. He called this time window a "critical period". Most notably, the gosling would imprint on Lorenz himself (more specifically, on his wading boots), and he is often depicted being followed by a gaggle of geese who had imprinted on him. Filial imprinting is not restricted to non-human animals that are able to follow their parents, however; in child development the term is used to refer to the process by which a baby learns who is his/her mother.

2.2 Sexual imprinting

Sexual imprinting is the process by which a young animal learns the characteristics of an appropriate mate. For example, male zebra finches appear to prefer mates with the appearance of the female bird that rears them, rather than mates of their own species.

3. METHODS

Before the practical the chicks are kept separately together with their own mock hens for a few days so that they can form a bond with it and chicks can get imprinted on their mock hens, learning their characteristics.

At the practical we work in groups of 2-3 students.

3.1 Tests

A. Separation test

Questions:

1. Do the chicks behave differently in the presence and in the absence of the mock hen?

It can be assumed that as a result of the imprinting the chicks will show an alarm reaction after the removal of the chicken. In natural circumstances the function of the alarm reaction is to search for the mother and to activate the searching behaviour of the mother. If we find difference in the chicks' behaviour with and without the mock hen it shows the sensitivity for separation from it.

2. Do the chicks behave differently in the presence of a familiar and an unfamiliar mock hen?

We assume that the chicks learn about the visual features of their mock mother (the mock hen) and so when they are in the presence of an unfamiliar mock hen, they show an alarm reaction as well.

Method

Before starting the experiment, observe the chicks' behaviour for a few minutes to be able to recognize the behavioural elements.

Behavioural variables of the chicks to observe:

1. *Standing*
2. *Walking*
3. *Breaking out*
4. *Contact vocalization*
5. *Alarm vocalization*

Phases of the experiment:

Do not forget to use the stopwatch during the test phases! The phases follow each other without break, there is no need to take out the chicks from the box between them.

1. phase: the chick is in a novel place together with the mock hen. **2 minutes**
2. phase: we get out the mock hen and continue to observe the chicks' behaviour for **2 minutes**.
3. phase: the chick is together with an unfamiliar mock hen for **2 minutes.**
4. phase: the chick is together with it's own mock hen again for **2 minutes**.

Write down the behaviour of the chicks in every other second with writing an x to the particular variable the chick is engaged in at the moment. Be careful that several behaviour elements can be performed at the same time.

Figure V.1. The coding sheet with hypothetical data for the imprinting test

IMPRINTING DATA SHEET					
Observer:				Date:	
Phase:					
Time (in seconds)	**Standing**	**Walking**	**Breaking out**	**Contact sounds**	**Alarm calls**
0	x				
2	x				
4	x				
6		x			
8		x			
10			x		
12	x				
14	x				
16	x			x	
18			x	x	
20			x	x	
.....					
Sum	6	2	3	3	0
Latency				16	20

Data analysis

For the statistical analysis we merge together the data of all chicks.

We assume that the chicks feel safe with their mock hens so their behaviour with the mock hens can be considered as a baseline and the following situations can be compared to this situation (1. phase).

According to the 0-hypothesis there will be no difference in the chicks' behaviour with and without their mock hens. To test this hypothesis we compare the chicks' behaviour between the first and the second phases separately for all behavioural variables. As we compare the behaviour of the same individuals we use repeated measures analysis. We are going to use Friedman ANOVA and/or Wilcoxon paired test. The same method will be used for comparing the chicks' behaviour with the familiar and the unfamiliar mock hens. For the statistical analysis we use the software „INSTAT".

For answering the second question of this study we compare the chicks' behaviour in the third and in the fourth phase (according to the 0-hypothesis there will be no difference between these).

Further task:

What questions could you ask for the comparison of the 2nd and the 3rd and for the comparison of the 1st and the 4th phases?

Discussion

Answer all the questions according to the points below:

1. Did you find differences in any of the variables? In which variables?
2. Do the results provide proof for the 0 hypothesis?
3. If the results provide support for other hypotheses, what is the alternative hypothesis, and what explanations could be given for the findings?
4. What is the reason if we do not find statistical differences?
5. What are the disadvantages of the used experimental procedure?

Note:

If we cannot find statistically significant difference then there is no difference in the behaviour of the two groups.

Wait 10 minutes between two tests.

B. Studying the following behaviour

In natural circumstances the chicks often follow their mother, but in the laboratory chicks do not have the possibility for exercising this behaviour. This way emerges the question whether the following behaviour can be evoked spontaneously or it is a learnt behaviour.

Questions

1. Can we evoke the following behaviour in unfamiliar environment?
2. Does the latency of the following behaviour change in time?

Method

1. We keep the chick gently at the end of a „running corridor" while we put the mock hen in a distance of 50 centimeters from the chick to the other end.
2. With a quick movement we let the chick move freely and start the stopwatch.
3. If the chick approaches the mock hen to a distance of 2 centimeters the trial is ended. (We write down the latency of both the leaving - when the chick starts to move from the start position - and of the arrival of the chick. Each trial lasts for 1 minute, if the chick does not approach the mock hen, the latency is considered 1 minute.)
4. We put the chick back to its box and wait 2 minutes before repeating the trial.

Repeat the same procedure 6 times.

Data analysis

We analyze the data on the group level, using Friedman ANOVA for comparing the latency among the trials.

Discussion

In the report describe the results on both the individual and on the group level. Answer the following questions:

On the basis of the results can we say that the following behaviour does not need previous experience? If there was difference among the latencies of the trials, what can be the reason for it? Do you think that the local environment has an effect on this behaviour?

C. Discrimination study

Question

1. Do chicks discriminate between their own mock hen and an unfamiliar one?

Method

1. We put the two mock hens at the two ends of the running corridor.
2. We put the chick to the middle of the running corridor in a way that it looks at the wall of the corridor (and not towards any of the mock hens).
3. We release the chick.
4. The test lasts maximum 1 minute or till the chick approaches one of the mock hens in a distance of 2 centimeters.

We put the chick back to its box and wait 2 minutes before repeating the trial.

Repeat the same procedure 10 times. Switch the unfamiliar mock hens 5 times (2 trials with each), and change the place of the chick's own mock hen in every trial (left or right side).

Write down the choice and the latency of the arrival in each case.

Analysis

Analyze the data both on the individual and on the group level. The chance level is 50%, which means that the chick has 50% chance in every trial to choose its own mock hen. We can test if the chick's performance differs from the chance level by using binomial test in the individual level and with Wilcoxon one sample sign rank test on the group level.

Discussion

1. Did the chicks differentiate between the mock hens?

2. What do you think; does the ability of discrimination depend on the features of the mock hen?

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Chapter VI. The effect of early human contact on the timidity of rabbits

Vilmos Altbäcker
Ágnes Bilkó

1. OBJECTIVES

Early decades of the development of ethology often involved hand raising animals which enabled scientists to make intimate observations on tame individuals. Lorenz and Tinbergen would have been unable to study the egg rolling of geese without raising goslings imprinted to humans and showing no avoidance during the later observations and experiments. Nevertheless, hand raising may also result in distorted behaviour if species specific forms of social responses cannot be learnt from conspecific partners. During this practical, we will study how handling, exposing the animals to several stimuli of human origin, affects the later responses of the handled animals to humans. We will thus compare the behaviour of both handled and non-handled individuals. We expect that handling will be most effective if applied in the sensitive period of conspecific recognition development of the rabbit. Data on the fear reactions will be recorded and groups of handled and non-handled individuals compared.

2. INTRODUCTION

The main goal of the domestication process is to eliminate unnecessarily strong fear responses (Price, 1984), but domesticated animals still show avoidance toward human beings (Rushen et al., 1999). Fear can be reduced by selecting the tamest individuals for breeding (Simm et al., 1996) but fear of humans can be further reduced by handling the animals in several species (Hemsworth, 2003). The handling procedure must be well timed; many mammal species have a sensitive period when handling is most effective. Goats handled in the sensitive period become tame and remain fearless even in adulthood (Klopfer and Klopfer, 1977). Tame animals are easier to work with (Boissy and Bouissou, 1988), they eat more (Day et al., 2002), develop faster and are more fertile (Coubrough, 1985) than timid animals.Lorenz himself started to study ducks but hand raising resulted in animals imprinted on him, regarding the researcher not only as their mother but also as potential sexual partner. Geese, on the contrary, have shown only maternal imprinting and this did not affect their later partner preferences. Therefore, the geese, unlike ducks, could be easily bred for developmental studies and these fearless birds were and still are favorite subjects of human-animal interactions.

Rabbit pups handled (held in the hand) around nursing were proven to be tame at weaning (Bilkó and Altbäcker, 2000). The procedure is efficient only if it is conducted in the first week of the pups' life and within 0.5 h after nursing. The tameness remains till adulthood (Pongrácz and Altbäcker, 1999), moreover, handled animals are more fertile than unhandled ones (Bilkó and Altbäcker, 2000). The effect of handling is very specific; when the pups were exposed to a tame cat, by placing the cat over the litters in the first week of the pups' life, they became tame only towards the cat, but not towards humans as well (Pongrácz et al., 2001). The duration of the daily treatment is not crucial, at least one minute of exposure to humans daily is enough and thus it can be integrated to intensive rabbitries too (Csatádi et al 2008).

2.1 Conspecific recognition of hand raised rabbits

The reduced level of fear of humans is durable as if handled rabbits tested at 6 month of age also showed reduced fear compared to non-handled ones. The behaviour of handled rabbits is similar toward humans to what can be observed when they meet their mothers. Contrarily, non-handled rabbits respond to humans similarly to what can be seen when they are exposed to a stuffed fox (Pongrácz and Altbäcker; 1999). Thus, handled rabbits may show an altered conspecific recognition which also includes humans as attractive objects. Nevertheless, the sexual preference of handled rabbits is not affected, they even show an elevated fertility compared to non-handled females. This difference might originate from the stress elicited by the being captured when taken to the buck for breeding in non-handled individuals (Bilkó and Altbäcker, 2000). The effect of hand raising is similar in geese, it only affects

the conspecific recognition in the goslings but sexual preferences remain unaltered as such preferences are formed later in life (Kotrshaal et al, 2005).

2.2 Conspecific recognition is based on smell in rabbits

Being nocturnal mammals, rabbits possess well developed chemical communication system. Young rabbit pups are able to recognize other individuals even if their eyes are closed (Mykytowycz, 1979). As the developing olfactory system of rabbit pups is most aroused and capable of olfactory learning during the maternal visits (Allingham et al., 1998) handling should be inefficient if it is conducted out of the nursing time or after the first week postpartum. Kersten et al. (1989) and Meisser et al. (1989) in their earlier studies handled animals beyond the sensitive period and did not find behavioural changes in the experimental animals. It is likely that pups learn thesmell of humans (Bilkóand Altbäcker, 2000), as their eyes are still closed in this period, and animals exposed to human smell without being touched also became tame.

3. METHODS

3.1 Experimental animals

Experimental subjects will be wild rabbit weanling pups kept at the breeding house of the ELTE Biological Station at Göd. The pregnant females were housed individually in standard wire-mesh cages (45 cm x 55 cm x 65 cm) with ad libitum pelleted laboratory food (Agrokomplex) and water. One day before the expected parturition does were provided with an outside plastic box and bedding. The entrance of the nest box was closed immediately after the mothers gave birth. The litters were culled to eight pups each; offspring were taken from does which gave birth to more than eight pups and were put into nests of does which had less then eight pups. Litters from naturally inseminated does were randomly assigned to treatment groups, pups of the handled group were weighed within 15 minutes after each nursing visits in their first week of life, while non-handled control animals were raised without human contact in this period.

According to the rabbits natural schedule (Hudson and Distel, 1989), does were allowed in the nest box to nurse only once each day in the same time until day 10 then the entrance was opened permanently. On day 28, the nest box was removed and the pups were weaned. At weaning, we place the animals individually into the 45 cm x 55 cm x 65 cm wire-mesh rabbit cage for 5 min to habituate. After this, the experimenter approaches the cage to within one arm's length, and places her hand against the mesh wall. The pup's location in the cage is not controlled for. Latency to the first approach by the pup (in seconds) and the total number of approaches are to be recorded during the 5 min test period. An approach is registered only when the pup touches the experimenter's hand. (see Figure VI.1.)

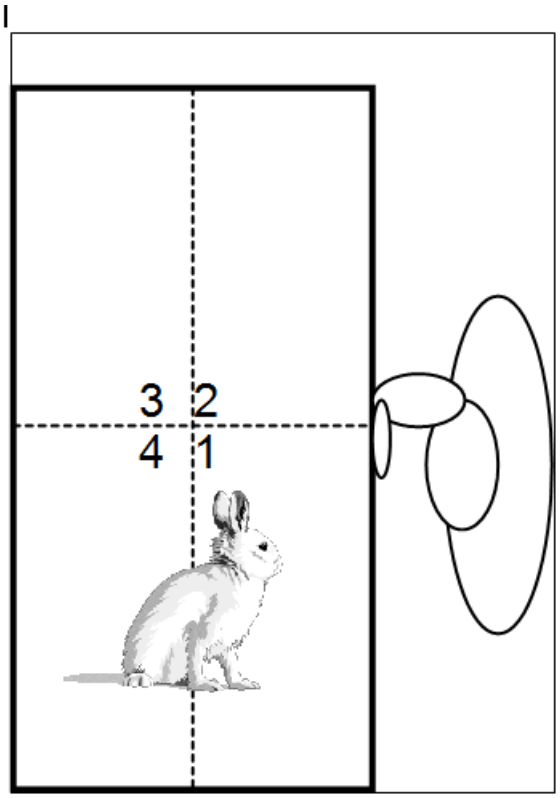

Figure VI.1 Schema of the open field arena to be used. The experimenter puts his palm at the middle of the cage's wall. The rabbit movement can be coded by locating its head to one of four labeled compartments.

4. STEPS OF THE PRACTICAL

During the previous tests, the subjects of an approach test in their empty cage just after weaning and at 6 month of age were video recorded. We will use these records in the present practical to compare the responses of handled and non-handled individuals to humans. After a short observation period to form initial impressions of the nature and extent of behavioural differences, we will design our study, define variables to be recorded, and prepare the data sheet for recording the behaviour of the animals.

We start the practical by discussing the main factors which may affect the responses of rabbits to human observers. Then:

1. We will get initial impressions on the variation of their responses when humans approach them in their new cage by looking at one from each group of the video footages.

2. Design the study by completing the design sheet (Fig 6.3., see later).

3. For this we start with formulating a question based on the overview of the initial impressions (e.g. Are handled animals tamer than non-handled individuals?
   - Decide the sample size by group, which is usually 7-7 as the minimum
   - Choose the variables to be recorded (see instructions later) and the length of the test.
   - Choose the statistical methods to be used, this is the Student t test for continuous variables, Mann-Whitney U test for frequency data.

4. You should record the occurrence of the following variables: a./ The latency time (s) to the first approach of the hand, b./ The number of approaches in the 10 min long test, and c./ the number of crossings from one field to any other (activity score).

5. After the data collection, you should enter the data to an Excel sheet.

6. Group means and standard deviations should be calculated.

7. Construct a Diagram showing the group means and standard deviations. Do not forget to label the axes.

8. Analyze the data by copying the data block from Excel to the Instat program. As we compare data for two independent groups, we will apply the Student t test. The results should be reported in the following way: The groups were / were not significantly different (t(df)=…, p=…..).

9. We answer the original question: yes the groups differed, or no, we could not find a difference between the two groups.

10. We discuss what have been learnt during the experiment. As we have recorded several variables, there must have been differences in either in the applicability, feasibility, accuracy or reliability of data obtained. Based on such experiences, make recommendations for later studies

11. Finish by formulating a new question to a future extension of the study.

Figure VI.2.Data sheet template

DATA SHEET for studying the effect of handling on rabbit timidity

DATE: **EXPERIMENTER:**

GROUP	VAR	UNIT	CODE	Lat (s)	Appr	Crosses	REMARK
1	handled	1					
1		2					
1		3					
1		4					
1		5					
1		6					
1		7					
1		8					
1		9					
1		10					
2	not handled	1					
2		2					
2		3					
2		4					
2		5					
2		6					
2		7					
2		8					
2		9					
2		10					
1	Mean group1:						
1	Mean group2:						
1	Standard deviation group 1:						
1	Standard deviation group 2:						

Stat type: result1 result2 result3

t(df)=…, p=….., t(df)=…, p=….., t(df)=…, p=…..

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Chapter VII. Study of chin marking behaviour in the european rabbit

Vilmos Altbäcker
Ágnes Bilkó

1. OBJECTIVES

We will observe and describe one form of chemical communication in the rabbit. This involves:

1. Investigation of chin gland and chin marking activity on living animals.

2. Study of sex difference in spontaneous chin marking activity (comparing female and male marking activity by coding it from video footages).

During the practical, chin marking activity of European rabbits originating from the breeding stock of the Department of Ethology will be compared, by describing the chin marking frequency of caged animals.

2. INTRODUCTION

2.1 Chemical communication in mammals

Most mammalian species live in complex social systems where communication among individuals is important. The signals are sometimes acoustic signs, or visual cues, but most frequently they are some type of odorants. From evolutionary point of view, the most plausible explanation for this is that the ancient mammals were nocturnal animals, where visual cues could play less important role than we perceive it today.

Nocturnal life rendered chemical signals more advantageous traits than the use of other communication channels.

- They can be easily detected even when visual or acoustic signs are not useful, during the night, under the ground, or in dense vegetation.
- Odours might provide exact information about an individual spatial or temporal movement.
- Chemical signals last much longer then visual or acoustic signals and remain effective even when the individual is far away.

Chemicals used by mammals are more sophisticated materials than insect pheromones (Mykytowycz, 1979). The mammalian substances are usually complex mixtures and the evoked behaviour reactions are more complicated too, compared to those of insect pheromones.

Brown (1979) states that the triggered behavioural reaction depends heavily upon the context and the previous experience in mammals, thus he prefers the expression “social smell” instead of the term ‘pheromone’. The signal might be the smell of the urine itself, as we can see in Canines, but certain chemicals in the urine may convey information on the identity or sexual status of the signaller, too. Animals might also use excretions of special skin glands as communicative signals. When the gland itself is situated near or around the anus, the excretion is mixed with faces or urine. In the European rabbit, excretion of the anal gland exerts as territorial marker on the surface of the faecal pellets (Mykytowycz, 1968).

Special skin glands however can be found on different parts of the body: infra-orbital region in deer, behind the eyes and both side of the jaw in the pika and in the woodchuck. In case of the European rabbit, there are three important skin glands, the chin, anal, and inguinal glands.

2.2 Sexual communication in the European rabbit

As a nocturnal animal, which is easily kept under laboratory conditions, the European rabbit is an optimal model species to study chemical communication and the role of chemical signals.

Young animals, even before postnatal day 10 when their eyes are opening, are able to recognise the conspecifics based on their smell (Mykytowycz, 1979). This phenomenon is quite reasonable, considering that this species spends two-third of its life in almost complete darkness: feeding during the night or resting underground during daytime. During the first 14-16 days of their life the offspring also meet with their nest-mates or mother only under complete darkness in the underground nest burrow. Under these conditions vision is not a useful way of obtaining information; however olfaction is of important value. The smell of a young animal might evoke interest from the mother, but it can induce aggression from another female of the same colony. Interestingly, a female might not recognise a strange offspring in the nest however she might kill it in other part of her territory outside the nest. A buck however tolerates and cleans an offspring independently of its origin anywhere in the territory (Mykytowycz 1979). The effect of the smell of an offspring also depends heavily upon the age of the animals and the actual situation due to interactions of young animals. Members of the same social group or the same nest might tolerate each other. However, the home territory does not provide defense anymore when they become 60-90 days old (the age of sexual maturity). At this time of age, they neglect territorial boundaries and aim to belong to a new group, despite that older individuals are quite aggressive with new intruders (Mykytowycz, 1979.)

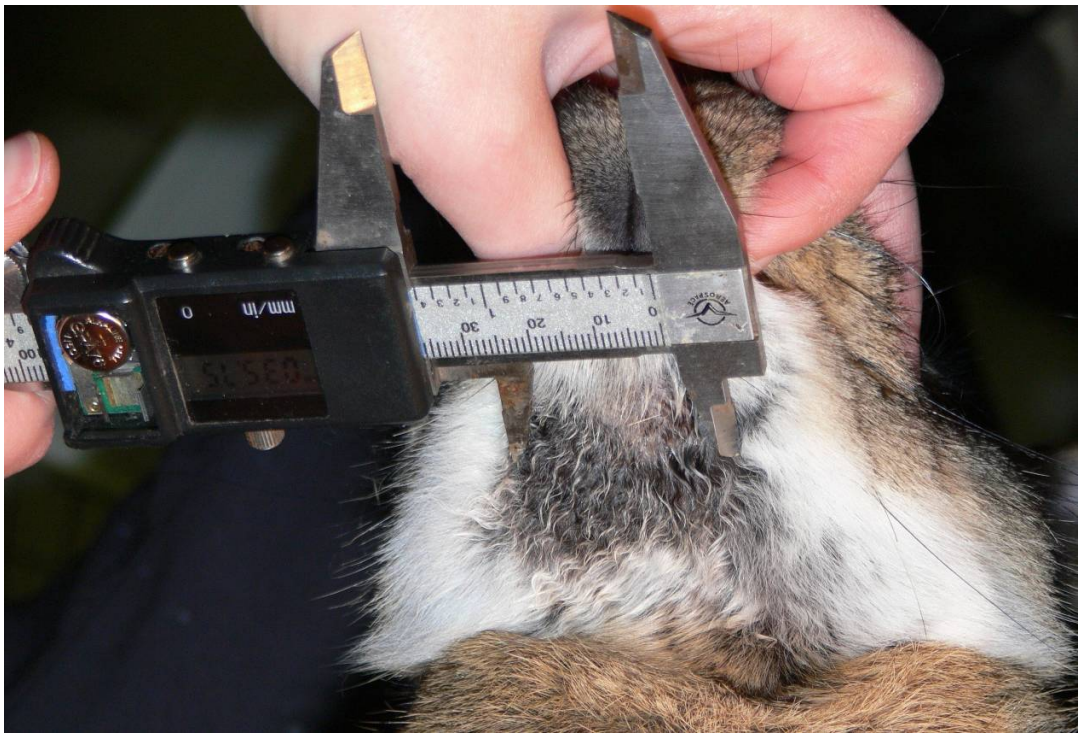
Underlining the importance of olfaction, excretion of several skin glands serves as communicative signals in rabbits. Excretion of the anal gland on the faecal pellets serves as territorial marker in the bucks. Faecal pellets are not deposited randomly but placed on special marking sites (called dunghills), especially in the breeding season (Förgeteg, 1991.) The dunghills are 1-1.5 m in diameter and are 10-15 m apart. Dunghills usually mark pathways within the territory and the most frequently used are those deposited on the territory border. These border hills are visited by the bucks of both adjacent territories (Mykytowycz, 1968).

The excretion of the inguinal gland plays role in individual recognition and provides information about sexual receptivity (Goodrich, 1983). When a dummy is labeled with the excretion of the inguinal gland, it evokes mating behaviour from the tested buck that repeatedly mounts the dummy (Robyn Hudson, pers. comm.).

2.3 Chin marking in the rabbit

Study of chin marking became an interesting area at the last eighties. Chin marking itself is the marking behaviour when the chin gland is actively rubbed against specific objects and the excretion is smeared on the surface. Both sexes have chin glands, although this gland is much more developed in bucks, both in size and in its productivity (see Fig 7.1.). This was the reason why primarily it was believed that this gland is only functional in the males. Mykytowycz interpreted that the marking by the chin gland in males serve as territorial marking, complementing the anal gland marking. It was supported by the finding that in bucks the size and activity of the chin gland correlated with the rank of the animal, mirroring the blood testosterone level and sexual activity of the individual (Mykytowycz 1965).

Figure VII.1. How to measure the diameter of rabbit chin gland



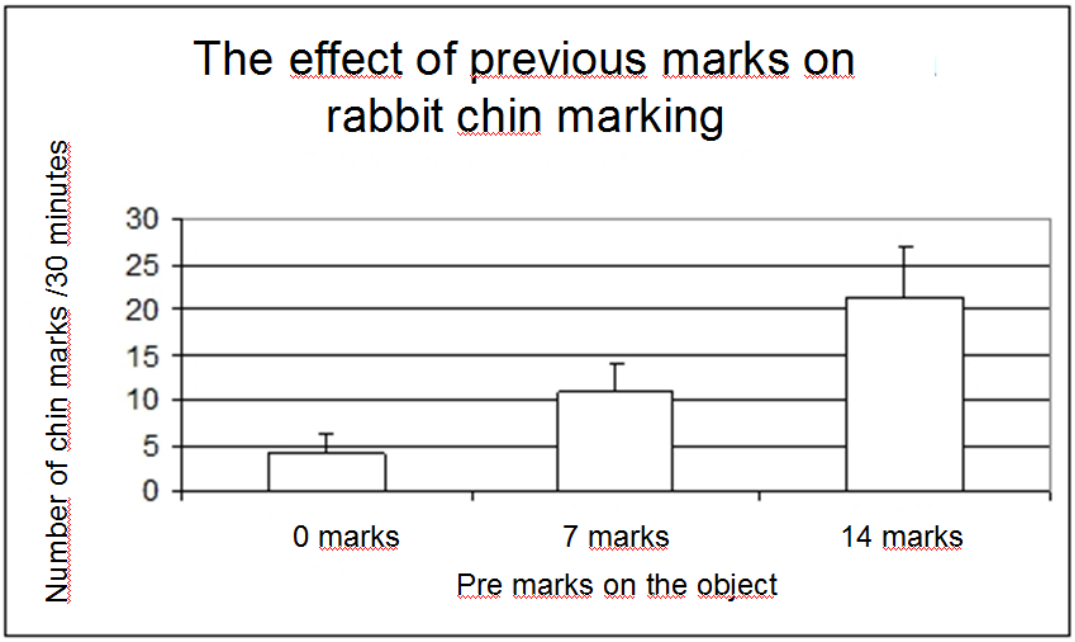
Chin marking was not so intensively studied in females, but it was found by Soares and Diamond (1982) that chin marking activity in females is in correlation with sexual status. Gonzalez-Mariscal and her co-workers (1990) investigated the spontaneous chin marking activity in female rabbits as a function of their natural sexual cycle. According to their method, the animals were put individually into a circular arena 1m in diameter, in which they found a brick as an object to mark on. The experimenters described chin marking activity by counting chin marks the animals placed onto the brick during a given test period. They investigated the animals daily during a 1.5 month period, then all animals were bred. Chin marking measures were continued during pregnancy, lactation and weaning period as well. According to their results, spontaneous chin marking activity strongly decreased after mating and remained low during the pregnancy and lactation period. The chin marking activity rose again to the original high level at the time of weaning the litter. However, if pups were separated just after the parturition, chin marking activity increased suddenly.

The role of sexual hormones in sexual cycle and in spontaneous chin marking activity was investigated by Hudson and her co-workers, in ovariectomized rabbits. They simulated the change of sexual status by administering different amounts of sex hormones to the does. During estrus, the level of estradiol was kept high, pregnancy was mimicked by a high level of progesterone in the blood, and parturition meant a drop in the progesterone level. The experimenters measured the spontaneous chin marking activity and willingness to mate during the experimental period. The results were similar to the natural situation: administration of estradiol increased the chin marking activity and willingness to mate. Administration of estradiol and progesterone together led to a marked decrease in chin marking activity and a sudden change in behaviour toward males. Sudden distraction of progesterone has led to a gradual increase in spontaneous chin marking activity, which reached the original level in 3-4 days. It is of special interest that spontaneous chin marking activity remained constant during the before the mating period, however it showed remarkable individual differences. This rises the question whether there is an estrus cycle in the rabbit or not, and if so, can it be predicted by measuring spontaneous chin marking activity?

There are additional factors affecting chin marking activity. This was investigated by Hudson and Vodermayer (1992). By keeping the animal under laboratory conditions and changing the day-length artificially, it was found that spontaneous chin marking activity increased by the increase of day-length and by keeping the animals under constant14 hours daylight-10 hours dark light regime. This was accompanied by a change in vulva colour as well. During long day condition, the vulva is dark red and enlarged, while the vulva colour is pale and the size decreases under short day conditions. It was found furthermore that chin marking activity is increased by the presence of chin marks from conspecifics.

Bricks pre-marked by females or males always increased the marking activity, although this effect was markedly different depending on the sex of the pre-marking animals. Females prefer to overmark the marks of male conspecifics. However, when the marked objects originated from diverse females, the difference in overmarking activity still remained. It was suggested therefore that chin marking might play a role in individual recognition as well. Another test showed that the number of pre-marks by other individuals affects also the marking activity (Figure VII.2.)

Figure VII.2. result of Dombay (1997) study focusing on the effect of previous marks



Goodrich and Mykytowycz (1972) investigated the composition of the different skin glands in the rabbit and found that the composition of the excretion of the 3 different types of skin gland was different. Chin gland secretion is a bit different from both the anal and the inguinal glands, as it lacks the free lipid components, thus it does not have the typical rabbit smell. Instead, it contains a high amount of non-volatile compounds with high molecular weight. The chemicals in the chin excretum are predominantly aromatic substances compared to the anal gland secretion, where long chained molecules are abundant (Goodrich 1983). Protein content of the secretions always differs, as this component is much diverse in type in bucks compared to females (Goodrich and Mykytowycz 1972). This difference can be explained quite easily by considering the completely different function of the scent in the two sexes.

2.3.1 What is chin marking?

When the animal actively rubs her/his chin against an object, this spreads the excretion of the chin gland onto the surface. In the laboratory, such object can be a brick, where the edges can serve as an appropriate surface to mark on.

How can you recognise chin-marking behaviour? During chin marking the animal intentionally puts its head on a given object ie. against the corner of the brick, and pushes it while the chin gland is rubbed against the surface. The length of this movement varies, sometimes it is just a sudden short motion.

The course of the practical:

1. each student should investigate the exact place, shape and size of the chin gland in male and female individuals.

2. we design the study by filling the form titled ” Necessary steps of a scientific investigation”
   - Start with a barkochba question regarding the validity of our initial observation
   - List possible answers (alternative hypotheses) to the question (yes/no)
   - Decide grouping variable (male, female)

- Consider possible variables to describe group differences in chin marking
- Define four variables (what to measure, equipment, how to use it, units of measurement)
- Decide group size, sampling procedure
- Decide which statistics is to be used for analyzing the data
- Construct the data sheet (do not forget to fill the header with your name and date of practical)

3. During the practical, everybody has to record how many chin marks were put in each minute of the 5 minute test session onto the brick on other place in the test arena. Additional variables can be: sex status, body weight, gland size, vulva color, etc

4. Data have to be typed in to an excel table matching the data sheet in its structure

5. Data should be analyzed by calculating averages and standard deviations

6. You should construct a bar chart in MS Excel showing the averages and standard deviations of chin marks by both males and females.

7. Data should be analyzed by using the Instat program. As we have two independent groups we will use t-test. The result must be given as t(df)=……, p=……,

8. Having the results, do not forget to give a clear, concise answer to your original question.

9. Discuss your results, compare your results from different variables to the results of at least your neighbour students and previous studies cited in the Literature.

10. Based on your conclusions, suggest a new question to extend the study. You may consider incorporating the age, pregnancy and hormonal status of the female, the presence of previous marks, etc.

You have to submit the original data sheet with all parts filled in at the end of the practical.

Figure VII.3. design of the rabbit chin mark study

Necessary and sufficient steps of a scientific study - research design template

1/ INITIAL (DECISIVE!) QUESTION:

2/ ALTERNATIVE HYPOTHESES (answers)
A:
B:
3/ EXP DESIGN: GROUPS:
LIST POSSIBLE VARIABLES:
DEFINE VARIABLES TO BE MEASURED:
Var1: name-equipment- how-in what quantity
Var2:
Var3:
Var4:
4/ SAMPLING DESIGN
-UNITS / GROUP: n=2V/D2
-SIZE of sample area/LENGHT of test
-distribution: random/even

5/ METHOD/EQUIPMENT TEST:
ACCURACY versus RELIABILITY

6/ CONSTRUCT A DATA SHEET:
header, signature, remark, data in one block

7/ OBTAIN DATA: it can save your life!

8/ ANALYSE DATA: A/ t; U; X2 TEST, B/ CORRELATION
INSTAT
9/ RESULT (answer) IN ONE SENTENCE:

10/ DISCUSSION:

11/ NEW, IMPROVED QUESTION:

Figure VII.4. data sheet for the rabbit chin mark study

DATA SHEET for studying.....

DATE: **YOUR NAME:**

GROUP VAR	UNIT CODE	Var1	Var2	Var3	Var4	REMARK
1	1					
1	2					
1	3					
1	4					
1	5					
1	6					
1	7					
1	8					
1	9					
1	10					
2	1					
2	2					
2	3					
2	4					
2	5					
2	6					
2	7					
2	8					
2	9					
2	10					

1 **Mean group1:**
1 **Mean group2:**
1 **Standard deviation group 1:**
1 **Standard deviation group 2:**

Stat type:	result1	result2	result3	result4

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Chapter VIII. The effect of warning coloration on zebra finch (*Taeniopygia guttata*) boldness

Ákos Pogány

1. OBJECTIVES

This practical aims at introducing and familiarizing with the main steps of a complete behavioural laboratory experiment investigating the effects of aposematic warning coloration. This communication signal is of universal importance across the animal kingdom. We apply the novel object boldness test, a simple and popular method used in experimental studies of animal personality. Our model species is the zebra finch, so the practice provides opportunity to gather experience as to how to handle and work with small songbirds. As the behaviour of the focal subject is likely affected by the presence of the observer during testing, we exclude direct observation by using a modern video surveillance system to monitor the experimental trials. Statistical analysis of the collected data will be carried out by using statistical software and we interpret the results in biological context.

2. INTRODUCTION

2.1. Theoretical background of warning colorations

Remaining unnoticed by predators - the majority of prey species follows this evolutionary tactic; individuals often concealing themselves by resembling to the background of the environment to escape becoming food. The intense selection pressure by predators shapes the morphology and behaviour of prey species. The same strategy can also be applied on the predators' side - sit-and wait predators conceal themselves and wait for their prey to approach and then strike on them.

However, there are numerous species that have taken a different evolutionary direction and instead of disguise, they seem to draw attention by their striking colours. When formulating his evolutionary theory of sexual selection, one of the greatest challenges to Charles Darwin was to explain eye-catching coloration expressed in clearly asexual contexts (Komarek, 2003). In most cases, sexual selection could be associated with intense coloration, which, in theory, would draw attention of conspecifics during competition for mating possibilities. However, this did not provide a satisfactory explanation for why caterpillars of various butterfly species have also often conspicuous colours. As much as he was convinced of the truth regarding his theory of sexual selection, Darwin had to admit that it may not be applied in case of larvae. Following the advice of Henry Walter Bates, Darwin turned with this problem to Alfred Russel Wallace, who joined Bates to discover the Amazon rainforests. Wallace, in his reply, outlined an entirely new concept: he suggested that the primarily function of striking coloration in caterpillars is not for communicating with conspecifics, but with potential predators. According to this hypothesis, possible prey items draw the attention of their predators using optical stimuli to their dangerous, inedible or poisonous characteristics. Therefore, Wallace, who among other things is also famous for recognising the principles of evolution independently of Darwin (forcing Darwin to publish his theory earlier than his original plans), and Bates have already recognized at the end of the nineteenth century that in certain situations **striking coloration** instead of concealment may be an evolutionary beneficial strategy. In the latter case, the colours function as **warning signals**. Darwin was impressed by Wallace's theory finding it 'brilliant' as it turned out from a response written to his research colleague (Komarek, 2003).

Subsequently, a number of observations were carried out in which predation success (prey acquisition) was investigated in light of the warning coloration of prey. For instance, in an experiment lizards were offered to choose between food items coloured with neutral or warning coloration. Observations carried out on the field and laboratory experiments both supported the hypothesis of Wallace (1871).

2.2 Aposematic coloration

The expression **aposematic** (from Greek, meaning: 'away' and 'signal') was first used by Poulton (1890) for striking, contrasting warning coloration. These usually include red, yellow or orange colours but lighter shades of blue and green are also frequent, and often are coupled with black to improve contrast (see Figure VIII.1).

The information conveyed by aposematic coloration towards potential predators and the environment in general is that the species expressing this signal has **biological weapons** at disposal that will be applied in case of emergency (e.g. a serious attack). The weapon arsenal is extremely diverse, but most often it is some kind of secretum. In terms of the predator-prey interaction, ignoring warning coloration may have various outcomes, stemming from unpleasant, disgusting taste (the least severe consequence, e.g. consuming ladybugs or various snail species) to death (the most severe consequence, e.g. consuming poison dart frogs or coral snakes). We should note that although in the present practical we focus on striking coloration, appearance is not the exclusive carrier of information when it comes to warning signs. Other characteristic behaviours of the species, e.g. movement, posture, sound or scent markings, can also function as warning signals.

The function of aposematism was tested by Gittleman and Harvey (1980) in an elegant laboratory experiment using chicken. Young birds were offered by bread crumbs that were previously painted blue or green by food dye. The birds consumed food items of both colours with pleasure. Consequently, quinine sulphate and mustard were added to make both blue and green bread crumbs unpleasant to a similar extent. The chicks were then divided into four groups, in each group, blue or green food was provided on a blue or green background. In research practice, such arrangement of treatments is called complete factorial design; both treatments (food colour and background colour) have two levels (blue and green), and researchers tested for all possible four combinations of treatment levels. The results of this experiment showed that subjects of the two research group that received bread crumbs on contrasting background initially found and consumed more food than birds which were given food that blended into the background. As time progressed, however, an opposite trend emerged, as individuals in the eye-catching, contrasting treatment groups consumed less and less food, whereas individuals in the camouflaged food treatment groups continued consuming at the same intensity. Comparing the total food consumption in the four groups revealed that chicks in the camouflaged food groups consumed overall more food than chicks in the contrasting food group. The striking coloration of food, therefore, contributed to the development of aversion (i.e. disgust, avoidance).

2.3 Aposematism and mimicry

The association of biological weapons and warning coloration has led to the evolution of various types of mimicry. **Mimicry** is the similarity of one species to another i.e. one species is indistinguishable in appearance, sound, smell or any other behaviour from the other (Figure VIII.1). Without being exhaustive, below we discuss the two most common types of mimicry and their effects on the evolution of coloration.

There are a number of similarities in the lives of the two scientists, the British Henry Walter Bates and the German Johann Friedrich Theodor Müller. Both of them spent a significant part of their lives in Brazil. In addition, both researchers independently recognized that many butterflies belonging to different species share appearance of an extraordinary resemblance. Wallace also paid a lot of attention to this phenomenon (he also experienced first-hand mimicry in the Brazilian rainforests), but it was Bates and Müller who developed and worked out in details two alternative explanations for the evolution mimicry. Both of these evolutionary explanations are based on the original function of warning coloration, and subsequent tests found support for both of them. Acknowledging their contribution to understanding mimicry, these two main types of mimicries are named after Bates and Müller.

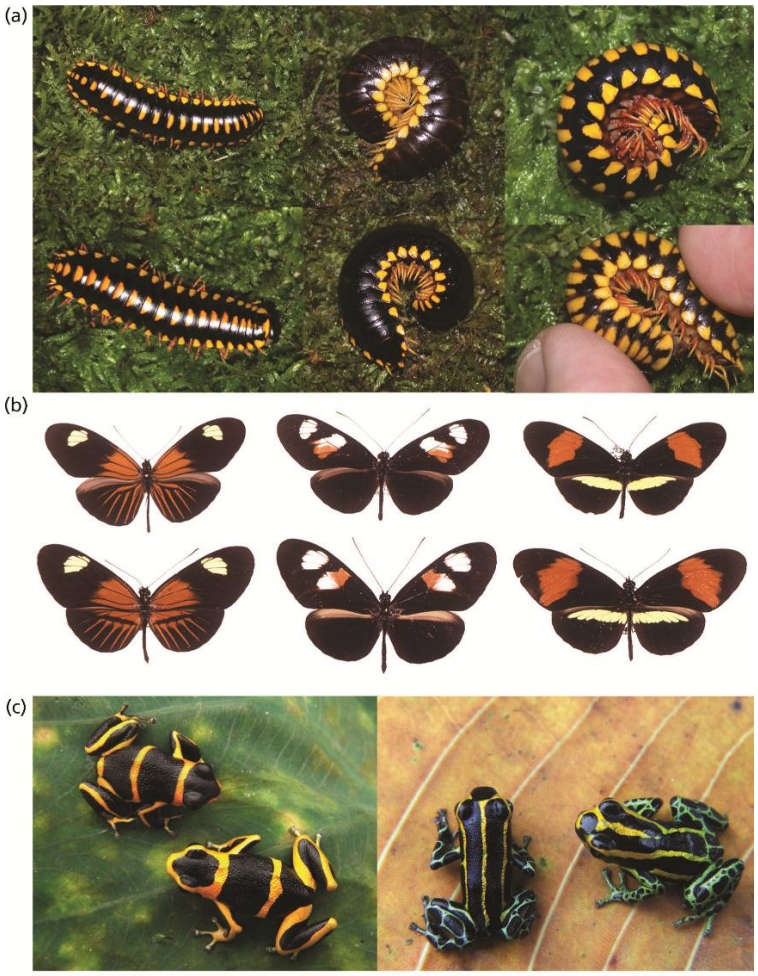

Figure VIII.1. Aposematism and Müllerian mimicry in the work of Merrill and Jiggins (2009). In all three examples, convergent evolution of distantly related species resulted in similar appearance of these species locally, whereas their coloration is variable across their range of distribution. (a) Apheloria millipede (top row) and its imitator Brachoria (bottom row) in three areas of their distribution. (b) Heliconius erato butterfly (top row) and its mimic H. melpomene (bottom row) in three geographic regions of the tropics. (c) Peruvian poison frogs in two geographic regions. Ranitomeya imitator (on the left in both photos) and its two mimics, R. summers (left photo) and R. ventrimaculata (right photo). @ photos: Paul Marek (a), Bernard D'Abrera (b) and Jason Brown (c).

a) Müllerian mimicry

The cooperative explanation of Müller (1878) for the evolution of similar species assumes that both species have their own biological weapons, to which drawing the attention of their predators is the common interest of these species. Müller worked out the following theoretical experiment in support of his argument; for a start, he assumed co-existence of two similarly poisonous species in a given area, one of them has a population size of 2,000 individuals (rarer species) whereas population size of the other is 10,000 (more abundant species). In addition, a constant population of a common predator lives in this area with 1,200 young, naïve individuals. These predators have never met any of their prey species before so did not have the chance to learn that they are poisonous. For simplicity, the example assumes that consuming one poisonous prey with warning coloration results in aversion (i.e. the predator will not try feeding later on this prey species in its life).

Starting from the above initial conditions, Müller first investigated the consequences if the two prey species have different coloration (i.e. there is no mimicry). If the two prey species are not similar, all young predators will have to consume one individual from both prey species for aversion to develop. Thus, numbers in the rare species will be reduced to 800 (the population will collapse), while the abundant species will be reduced to 8,800 (an acceptable loss). In contrast, if the two species are similar in appearance, predators will consume one individual from their common population of 12,000 individuals, 1.200 prey items in total. An important aspect is that the 1,200 consumed prey items consisted of two species at random, i.e. in proportion to their original population size (in our example,

1:5). Thus, the rarer species lose 200 individuals (an acceptable loss with 1,800 survivors), and the more abundant species has also lower loss, only 1,000 individuals.

The conclusion from Müller's elegant mathematical example is that this 'cooperative' type of mimicry is especially beneficial to the less abundant species, but fewer individuals become prey also from the more abundant species. In addition to the examples shown in Figure VIII.1, widespread examples of Müllerian mimicry include the yellow and black stripes of bees and wasps.

b) Batesian mimicry

While in Müllerian mimicry both species have harmful biological weapons and their similarity is mutually beneficial to both of them, Bates (1862) described a different evolutionary scenario in which a poisonous species (model) is copied by a harmless species (the mimic). To which of the two species and to what extent this type of mimicry is beneficial are less clear and straightforward as in case of Müllerian mimicry.

The evolution of coloration takes different directions in Müllerian and Batesian mimicries. This is because while the similarity of the two species is mutually beneficial in Müllerian mimicry (although proportionate to the relative abundance of the species, see above) and selection results in convergent evolution and increasing similarity, in Batesian mimicry, the benefits are highly frequency-dependent and it can be beneficial to switch model species for the mimic. In Batesian mimicry, therefore, the higher is the abundance of the model species and the lower is the abundance of the mimic species, the more profitable the mimicry is for the harmless species. However, as the mimic becomes more abundant due to taking advantage of the similarities, potential predators encounter them more often and consume them without any negative effects. This decreases the reliability of the signal, and the consequent costs are paid by both species. It also facilitates polymorphism in the mimic, as a mutant that is similar to another harmful model has significant advantages (assuming that the new copied signal is an honest one). Predation pressure on the model species also increases with more mimics in the population; however, the model cannot change coloration as any mutant would be fast predated. Neither their very low abundance, nor their new coloration (that predators have not yet learned to avoid) facilitates spreading of the new morph. Two common examples for Batesian mimicry are the wasp-like patterns of Syrphoidea flies, and the similarity between coral snakes and false coral snakes.

2.4 Animal personality and boldness

Various behaviours of individuals belonging to the same population often show great variation on population level, but also high consistency within individuals over time or different contexts. Some individuals are consistently more adventurous, curious, react to changing situations rapidly, while others are timid, cautious and slower to respond. Such consistent individual differences are referred to as **animal personality** ('behavioural syndrome' and 'temperament' are also frequently used terminology; Gosling, 2001). Similarly to human personality, animal personality has also different dimensions. One of the most widely studied dimensions is the shyness-boldness continuum i.e. how animals cope with stressful situations; and what is the degree of their risk-taking (Wilson et al., 1994).

Two of the most frequently used experimental approaches to testing boldness personality are the 'open field' and 'novel object' (or 'novelty') tests, that show high levels of consistency when carried out on the same individuals (Verbeek et al., 1994). In open field tests, the subject is placed into an unfamiliar environment (such as a new room), and the time needed to discover the new environment is measured (e.g. time needed to visit all ten perches by the subject bird) or we monitor the number of objects explored within a given time (e.g. how many perches the subject visited within 10 min). In novel object tests, the subject is tested in its usual environment, but we place an unfamiliar object in it and observe the latency to approach or touch this novel object.

3. MATERIALS

3.1 Experimental population and methods

This practical will use the zebra finch population kept at the Animal House of Eötvös Loránd University. Novel object boldness tests will be recorded on a digital video recorder. Following behavioural coding of video recordings, results will be analysed statistically using Instat statistical software.

4. PROCEDURE

4.1 Aims

We will study the effect of aposematic colorations by novel object boldness test. Zebra finches will be allocated to three experimental groups, and following food deprivation for 2 h, feeders will be replaced with a small alu (neutral) or yellow-black striped (aposematic) flag attached. Feeders of the third experimental group (control) will be replaced with no flag attached. Latency to the first feeding will be measured with the help of video recordings and compared between experimental groups.

4.2 Experimental steps

1. Asking research question, formulating hypothesis and predictions together.

2. Introducing the applied statistical method. Because our latency data will likely have non-normal distribution, our descriptive statistics will include quantiles and boxplot charts will be drawn. We will focus on two experimental groups (alu vs. striped flag) and their latencies will be compared by Mann-Whitney U-test.

3. Acquiring management of video surveillance system.

4. Novel object boldness test in the Animal House. Tests will be carried out in study groups of two, and every pair will test one bird. The feeders of food-deprived birds will be placed back, and we start recording the birds' behaviour for 1 h. Data to be included in the report:
   - cage no. of subject;
   - sex;
   - flag type (alu, striped or control);
   - start of test (this is the same for each pair: replacing the last feeder and leaving the room + one min).

5. Behavioural coding of videos and data entry. We play back digital videos using computers, with double playback speed and record the latency to first peck at food. We compile one excel dataset from the observations of workgroups.

6. Statistical analysis using Instat software. Analysis will be carried out alone using the compiled dataset that includes all observations. Data to be included in the report:
   - median (min-max) values of latencies of each experimental groups;
   - Mann-Whitney test statistic, degrees of freedom and significance level.

7. Conclusions of the experiment and discussion from biological aspect. Criteria:
   - Do our results support our hypothesis?
   - Can we answer our research question based on the statistical analysis and the data collected? (If not, suggest how to improve this experiment so that it would be suitable for answering reliably this question.)
   - Are there any alternative explanations that are in line with our results?
   - How would you move on? Ask novel research questions based on our findings!

8. Writing report.

Every student writes his/her own report (latency data for workgroup members are identical).

Figure VIII.2 Report sheet for the aposematic coloration practice

REPORT ON THE EFFECT OF APOSEMATIC COLORATION

Name: ..... **Group:** ..... **Date**:.....

Research question: .....

.....

Hypothesis, predictions:

H: .....

.....

P: .....

.....

Methods: .....

.....

.....

Data (behavioural response of one bird per study group of two students)

Cage no.:..... Sex:..... Flag:..... Start:..... Latency:..... min

Statistics (full dataset including all birds)

Group	Latency (min)		
	median	minimum	maximum
Alu			
Striped			

Test statistic: M-W *U*=..... *d.f.*=..... *p* =.....

Discussion of results and conclusions (continue on the back if necessary):

.....

.....

.....

.....

.....

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Chapter IX. Human sexual dimorphism

Tamás Faragó

1. OBJECTIVES

The main objective of this practical is to introduce the students to the methods of data-collection via measuring variables and analysis of such data. This will happen through practicing measurements of human sexual dimorphism. During the theoretical introduction the students will learn about the phenomenon of sexual dimorphism, mechanisms of sound production and how the sexual selection potentially affected the human dimorphic traits. In the actual practical part, the students will measure a couple of chosen bodily traits (height, shoulder-hip-waist circumference, 2nd and 4th digit length) on each other, plus additionally the demonstrator records their voice and performs measurements on the vocal parameters (pitch, formant frequencies). Besides these the students are also encouraged to suggest parameters for measuring. The collected data are pooled with the database of the earlier years' measurements, and this large dataset is used for statistical analysis. The students will compare the measured parameters between the two sexes, and also search for possible relationship between selected parameters within each sex.

2. INTRODUCTION

2.1 Background

The phenomenon of morphologic and behavioural differences between sexes of the same species was one of the major interests of Darwin (Darwin, 1874). The Darwinian concept of sexual selection explains the emergence of sexual dimorphic traits that cannot be a result of the process of natural selection. Sexual dimorphism is a direct consequence of anizogamy that appeared through the evolution of sexual reproduction (Parker et al., 1972). Both the role and the investment to the offspring in the two sexes are different during mating and producing offspring. Together with the fact that the two sexes are usually present in equal numbers in the population this can cause a conflict between the sexes and evoke the development of sexual selection (Krebs and Davies, 1993). Females produce a relatively low number, but large and costly gametes (eggs) that requires larger investment than the production of the males' numerous, small and agile gametes, thus males can fertilize eggs with a faster rate than the eggs are produced (Nakatsuru and Kramer, 1982). Moreover, physiological differences between the sexes can result that they are able to invest to their contribution to raise the offspring: for example, in mammals due to the internal fertilization, the father can easily desert, while the female is forced to invest in the development of the foetus. As a consequence of this, males will compete for females to be the father of as many offspring as possible, in contrast, for being successful during the reproduction a female should be choosy and prefer to mate with the higher quality male(s) only (Krebs and Davies, 1993). In contrast, among birds there is a higher ratio of monogamous species, due to the females' have the opportunity after laying the eggs to desert and leave the males to incubate. Finally, sexual selection causes competition between the individuals of the same sex, which eventually will lead to visible intrasex morphologic and behavioural differences, as these traits are supposedly correlated with the quality of the individual.

Sexual selection operates through two main mechanisms: it can be the competition within one sex for monopolization of the members of the other sex, or obtaining exclusive mating opportunities (intrasexual selection); or it can manifest itself in the selection between sexes, that is, members of one sex choosing their potential partners (intersexual selection) from the members of the other sex. Of course, the two phenomena are not working in complete isolation, often their parallel presence is responsible for the development of sex differences (see, eg. deers).

2.1.1 Female preference

Although female preference was considered for a long time far less significant than male-male competition, today its role is clear in sexual selection, and the factors that influence the females' decision are the most studied aspects of it (Krebs and Davies, 1993). It is known that females are picky, the reason for this lays in the production of small number of large eggs, and in some cases the incubation and parental care also takes much more effort for them, thus their interest will be to select the best from the available males. The benefit from the selection of a good

male can occur in several ways: it can be either genetic or non-genetic. The non-genetic benefit on one hand can be derived from the males that own territories of different quality (Møller and Jennions, 2001). In this case choosing of a male that is dominating over rich resources, or an area free of predators and parasites promises clear benefits for the females. On the other hand, for a female the foraging ability of a male can be also an important aspect to consider, because if the male is able to provide more food to the female and the offspring, that can greatly increase the chances of survival. Naturally, the non-genetic benefits may be closely linked to genetically transmissible properties (better physique → better territory) thus, these two factors are often not, or difficult to be separated from each other.

There are results that support also the existence of genetic gain through female choice. For example, when the female fruit flies (*Drosophila melanogaster*) were provided a random pair, their offspring had shorter survival time, in contrast if they would choose their mates themselves (Partridge, 1980). This suggests that female choice for a preferred male can lead to offspring with better genetics. The background mechanism of female choice can be explained by two classical hypotheses: either "good taste" or the need for "good genes". Darwin coined the former view, resolving the contradiction that in some species members of one sex carry features that are disadvantageous (may pose a serious risk for survival), so their appearance cannot be explained by natural selection. In his view, if the males carry certain features, such as the size of the nose that is more attractive to females, thus males with a longer nose will be more successful in breeding, and during the course of evolution, the nose size will gradually grow enormous. Of course, this is not a real sense of beauty, the female preference may be caused by simple sensory shift (some colours are more visible), that is a genetically determined character as well as the male's corresponding feature. According to Fisher (1930), females gain indirect reproductive benefits if they choose the most attractive males, because their male offspring will inherit the father's feature, and they will be able to produce greater number of grandchildren ("sexy son" hypothesis).

It is also conceivable, however, that these individual traits are not only exaggerated due to random preference, but they are actual indicators of male quality. Especially when producing such features is associated with high energetic costs, so the higher quality males can have more prominent, marked traits. In this case, the preference of the females for this character is formed because the features are the honest indicators of good genes ("good genes" hypothesis). Plenty of such features occur in the animal kingdom, for example the songs of the male songbirds, body size of mammals and other secondary sexual characteristics. In many cases a correlation can be shown between these characteristics and the physical condition of the male, its rank position, or its resistance against disease and parasites. However, according to Zahavi, these exaggerated traits after a level become an actual disadvantage to of the males, so those certainly carry very good genes, which can survive in spite of this handicap (handicap hypothesis: think for example peacock's tail), thus, the females can only benefit from mating with such males (Zahavi et al., 1997).

2.1.2 Male competition

The males are not idle either when it comes to breeding. Their success can be maximized if they manage to get more females (or eggs) fertilized (although, when the fathers care of offspring provides significant advantage for their survival, their preference for polygyny will drop). For this, however competition with each other is inevitable. This can manifest in actual physical combat (including the phenomenon of sperm-competition which is not lacking real fight) or ritualized behaviour (Krebs and Davies, 1993). In species where physical competition is typical, significant size difference can appear between the sexes, and these species can also carry a variety of weapons (antlers, horns, fangs) as well, which are mostly used during the male-male fights preceding the mating. (Of course, these may be useful for example for protection against predators, so they can enjoy female preference too. But in this case usually the females also bare these weapons)

Physical confrontations, because they are costly to both sides, and even can result in death, are often preceded by ritualized posturing and behaviours suitable for assessment the physical condition of the opponent. For example, red deer roars has a number of parameters, which contains information about the physical condition and body size of the stags (Clutton-Brock and Albon, 1979) (these parameters are monitored by the females too, and they show a preference for the stags with a roar that reports of a larger body size, so this selective pressure from the choosiness of the hinds affects the males too). Based on these, the challenger can decide if it is worthy to try to challenge the harem master or not. During the challenge visual cues are helpful, too, for example during the parallel running the stags assess each other's antlers, and musculature. Actual combat usually occurs only between stags with a very similar physical constitution.

2.2 Sexual dimorphism in humans

2.2.1 Secondary sexual traits

Sexual dimorphism can be observed in the case of humans too, men and women, in addition to their primary sexual characteristics show visible and measurable differences, including body size, body proportions, sound parameters, hairiness, body fat and its distribution, and in many cognitive abilities. These properties are called secondary sexual characteristics. The development of these differences is due to the effects of sex hormones, while in evolutionary time, the mechanisms of sexual selection is likely to have shaped these gender differences. In the following paragraphs some of these characters will be described in more detail, which will be the subjects of our study during the practical.

2.2.2 Voice as sexual signal

Voice production

In order to understand in what measurable parameters can differences be found between men's and women's voice, we must first get acquainted with the process of mammalian phonation. Currently, this is described most comprehensively by the so called source-filter theory (Fant, 1960). According to this, the vocal apparatus can be divided into two major functional units: the Source and the Filter. The energy required for voice production is provided by the movement of the breathing muscles in the chest, which pushes out the air from the lungs. The stream of air flowing through the bronchial tubes gets to the larynx, which is the actual place of sound production. The larynx, in essence is a sound box consisting cartilaginous elements, in which elastic mechanical vibrators, the vocal cords are located. These all together are called the source, because the sound wave produced here, will be the basis of the emitted vocalization. The cricoid cartilage gives the basis of the larynx and to this the thyroid cartilage and the two arytenoid cartilages are connected with joints. The vocal cords extending between the two arytenoid cartilages and the thyroid cartilage, at resting state are ensuring the free flow of the air. However, during phonation the contraction of the muscles stretching between the cricoid cartilage and arytenoid cartilages, will rotate and stretch the vocal cords pulling them into the cavity of the larynx. The vocal cords are rather tongue-like than ribbon-like structures that lay together blocking the path of the airflow. This leads to a pressure increase under the larynx, which lasts until it reaches a threshold, which is able to separate the vocal cords. Then the air flow starts again abruptly, this in turn will cause a decrease in the pressure, which together with the elasticity and inertia of the cords results in their collapse together, and then the process restarts. This cyclic change in pressure in the flowing air will be the sound wave itself. The cords' opening and closing frequency will give the fundamental frequency of the produced sound, which is essentially the pitch of the vocalisation. This frequency is mainly depends on the tension state of the vocal cords, and their weight and thickness. Since men have an enlarged larynx developing during the puberty due to the testosterone peak and their vocal chords thicken, the fundamental frequency shows sexual dimorphism (male mean: 120Hz, female: 220Hz) after maturation.

The sound waves that were formed in the larynx, at first pass through the upper part of the larynx, throat, the oral and nasal cavity before reaching the environment. These anatomical structures are called together as the vocal tract, forming the functional unit of the filter. The filter is basically an elastic-walled tube filled with air. This air column has so-called resonance frequencies, which can easily move the particles in the column, while other frequencies are muted. Thus, the vocal tract practically acts as a band filter that enhances certain frequency ranges, while suppresses others in the spectrum of the sound wave produced in the larynx, creating the final sounding of the vocalizations. The frequency bands that are strengthened by the filter are called formants. The location of these in the spectrum and the spacing between them primarily depends on the vocal tract length: the longer the vocal tract, the lower the formants positioned and have narrower intervals. As the vocal tract length is closely related to the anatomy of neck and skull, which in turn is mainly dependent on body size, we can say that the formant locations (especially their dispersion) can be a good indicator of the body size. In addition, during the maturation of the human males their larynx descends, causing an extension of the vocal tract, so the males can have much lower formants and lower formant dispersion than women (Huber et al., 1999). Must be added, however, that the great mobility of the human vocal apparatus during speech (which allows the production of diverse speech sounds) makes the examination of the formants difficult. In addition, the production of vowels happens by changing the first three formants' position, causing the formant dispersion being an unreliable cue for body size in humans (Gonzalez, 2004).

2.2.3 Body size as sexual character

Physical parameters can be also characterized by sexual dimorphism, men are being significantly heavier and taller than women (of course considering the fact that there is also a big difference among diverse populations of humans). Depending on the racial variance, men are taller after puberty by an average of 12-15 cm than women (Eveleth and Tanner, 1990). Body size dimorphism can be observed among primates, where gender difference is closely related to the degree of polygamy. While in the solitary and monogamous species (e.g. gibbons, night monkeys) the difference between the two gender is small, in polygamous species (macaques, chimpanzees) the males are 1.2-1.5 times larger than females, and in large harem holding species (baboons, gorillas) the male weight can be up to twice of the females' (Krebs and Davies, 1993). It is worth to note that in the case of chimpanzees the observed difference is significantly smaller than what would be expected from their group composition, suggesting reduced competition within males. However, when you consider that chimpanzee males have very well-developed penis and testicles to their size, it becomes clear that the likely occurrence of sperm competition have greater importance than physical clashes among the males. Humans can also be fairly well integrated into this line where based on the observed, rather slight dimorphism, the monogamous trend is more robust. Of course, we should not forget that environmental and cultural factors can also cause measurable difference between the sexes (Kanazawa and Novak, 2005).

2.2.4 Body ratios

Waist-to-hip and waist-to-shoulder ratios

Physical parameters can be also characterized by sexual dimorphism, men are being significantly heavier and taller than women (of course considering the fact that there is also a big difference among diverse populations of humans). Depending on the racial variance, men are taller after puberty by an average of 12-15 cm than women (Eveleth and Tanner, 1990). Body size dimorphism can be observed among primates, where gender difference is closely related to the degree of polygamy. While in the solitary and monogamous species (e.g. gibbons, night monkeys) the difference between the two gender is small, in polygamous species (macaques, chimpanzees) the males are 1.2-1.5 times larger than females, and in large harem holding species (baboons, gorillas) the male weight can be up to twice of the females' (Krebs and Davies, 1993). It is worth to note that in the case of chimpanzees the observed difference is significantly smaller than what would be expected from their group composition, suggesting reduced competition within males. However, when you consider that chimpanzee males have very well-developed penis and testicles to their size, it becomes clear that the likely occurrence of sperm competition have greater importance than physical clashes among the males. Humans can also be fairly well integrated into this line where based on the observed, rather slight dimorphism, the monogamous trend is more robust. Of course, we should not forget that environmental and cultural factors can also cause measurable difference between the sexes (Kanazawa and Novak, 2005).

The ratio of the Index and Ring finger length

Less obvious, but easily measurable sexual difference indicator variable is the index and ring finger length ratio (2D 4D). This feature is primarily depends on the ratio/amount of male and female sex hormones exposure of the foetus in utero (Zheng and Cohn, 2011). During the development of the fingers both androgen and oestrogen receptors have an important role, and these receptors are present in higher quantities in the ring finger. Since the androgens have stimulating effect on cartilage growth in the fingers, whilst the oestrogen acts as an inhibitor, thus in males the fingers are stretched more. Due the increased number of receptors on the ring finger it is more sensitive to sex hormones, so in males the ring finger is longer than the index finger, while in the women it is shorter or the same length (Figure 1). Accordingly, the male finger ratio is below 1, while the female is equal to 1 or higher. As the amount of sex hormones before birth affects postnatal development, the finger ratios has correlates also with observed physical, cognitive, behavioural and sexual traits (Williams et al., 2000; Ferdenzi et al., 2011).

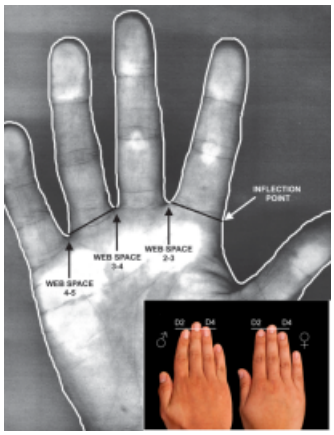

Figure IX.1 The human index finger- ring finger ratio sexual dimorphism and the required measurement points. (the original figure: (Zheng and Cohn, 2011) and (Nelson et al., 2006))

3. MATERIALS

3.1 Subjects and equipment

The students attending to the practical will be the subjects of the tests as well. At the beginning individuals will be assigned to perform particular measurements. Recording the body measurements strings and measuring tape will be used; the length of the fingers will be measured with the help of paper, pencil and ruler. Sound recordings are made with a PC connected handheld recorder (H4N) and Sennheiser ME66 shotgun microphone. The voice parameter analysis will be done with the linguistic software Praat, using a specially written script for this purpose. Data collection is done on paper datasheets, data analysis will be performed with computer, Excel and Instat software.

4. PROCEDURE

4.1 Aim of the study

During the practical, we will examine the sexual dimorphism in humans based on certain well-measurable traits. The primary question is that whether we find difference between the sexes in each features, and also to examine whether a relationship can be found among the features within the sexes, that may provide indirect information on the quality of individuals (e.g. ratio of male and female sex hormones).

4.2 Steps of the study

The measured variables are certain body dimensions (height, waist-to-shoulder, waist-to-hip ratio, index finger, ring finger ratio etc) and acoustic parameters (pitch, formant dispersion). We will discuss these before the beginning of data collection, and agree about their accurate measurements. During the data collection is important to record all the variables together for each subject, as it will allow correlation studies to be carried out.

The recordings and analysis of acoustic parameters will be done by the demonstrator, during this each students goes to the prepared sound recording equipment, and provide three "E" vowels held for 1-2 seconds into the microphone. During the vocalisation it is important to pay attention for keeping the pitch of normal speech, and applying sufficient volume.

Measurements of the body size will be done by volunteering students after sufficient practice. The height is measured without shoes, standing in the doorjamb, measured from the highest point of the crown, with the assistance of a set-square. The shoulder, waist and hip circumference is taken with a good length of string. The shoulder and hip are measured at their widest, and the waist at its narrowest part. The measuring of the length of fingers on the right hand starts by drawing the outline of the hand on a paper, and then the inflection points are connected around

the base of the ring and index finger. Midpoint of these sections are connected with the finger tips and the length of the fingers are measured (Figure 1). The data sets are recorded individually, on the datasheet which are provided by the demonstrator with a numerical code for each student individually. This ensures the protection of the students' privacy rights and keeps the individual data together.

The collected data are added onto an Excel sheet and bulked with the data had been collected in previous years. This makes it possible to achieve a sufficiently large number of subjects for the analysis (as well as to compare data between years). The demonstrator then presents the process and the statistical methods of data analysis with some examples.

4.3 Preparation of the study notes

Each student prepares the study note individually, based on the analysis and evaluation of the results of the released data from Excel spread sheets.

The record shall include the following details:

- Steps of a scientific investigation
- Completed copy of the Datasheet with filled letterhead (of course, the data series does not need to be attached)
- Results:
- A short introduction in which the theoretical background of the study is summarized
- Questions and Hypotheses
- Method, description of the measured variables and measurement procedures
- A summary of the comparisons (statistical method used)
- Comparison of the two groups for all measured variables (six comparisons)
- Statistical results (numerically, t and p-values and degrees of freedom are shown, and in the text also being presented with one or two sentences)
- Graphical presentation of the Results (Excel graph)
- Analysis of the relationship between some variables within groups with correlation tests (three variable pairs preferably biologically plausible, and relevant for answering our questions chosen in both groups. Six comparisons.).
- Results (p and r values) in short, full sentences
- Graphical representation of the Results (also Excel, scatter plots with trend lines)
- discussion of the Results up to one page (do the results meet with our hypotheses, if do not why not, etc), biological background, possible explanations for the phenomenon, the possibility of further studies.

The notes' preferred form is made with text editor, printed, stapled as a manuscript, but a neat hand-drawn/written version is also acceptable.

Fig 9.2 Datasheet for documenting human sexual dimorphism

Observer:.....Date:.....

Group	ID	gender	age	height	shoulder	waist	hip	2D	4D	F0	F1	F2	F3
	1												
Group	ID	gender	age	height	shoulder	waist	hip	2D	4D	F0	F1	F2	F3
	2												
Group	ID	gender	age	height	shoulder	waist	hip	2D	4D	F0	F1	F2	F3
	3												
Group	ID	gender	age	height	shoulder	waist	hip	2D	4D	F0	F1	F2	F3
	4												
Group	ID	gender	age	height	shoulder	waist	hip	2D	4D	F0	F1	F2	F3
	5												
Group	ID	gender	age	height	shoulder	waist	hip	2D	4D	F0	F1	F2	F3
	6												
Group	ID	gender	age	height	shoulder	waist	hip	2D	4D	F0	F1	F2	F3
	7												
Group	ID	gender	age	height	shoulder	waist	hip	2D	4D	F0	F1	F2	F3
	8												
Group	ID	gender	age	height	shoulder	waist	hip	2D	4D	F0	F1	F2	F3
	9												
Group	ID	gender	age	height	shoulder	waist	hip	2D	4D	F0	F1	F2	F3
	10												
Group	ID	gender	age	height	shoulder	waist	hip	2D	4D	F0	F1	F2	F3
	11												

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Chapter X. Human sexual selection: female and male preferences

Tamás Faragó

1. OBJECTIVES

The main objective of the practical is to show to the students the methods of questionnaire data collection and analysis. This will be done through measuring human sexual preferences. During the theoretical introduction the students will learn about the mechanisms of sexual selection, male and female competition and the peculiarities of human sexual preferences, and the types of questionnaires. In the practical part, the students will construct the questionnaire with the lead of the instructor. Besides the scales necessary for measuring preference (masculinity-femininity, dominance, attractiveness), the students can also suggest interesting parameters to measure. After this, the students will listen to and rate a selection of human sounds originating from the earlier years' Dimorphism practical. During the analysis they will prepare the dataset supplemented with the bodily and vocal parameters for statistical analysis, and compare the preference of the two sexes, and search for relations between the measured preference scales and the dimorphic vocal and bodily parameters.

2. INTRODUCTION

2.1 Background

As we have seen in the previous chapter one of the main driving force behind the development of sexual dimorphism can be the preference shown during mate choice for certain traits; and the competition within sexes. Of course, the two phenomena may be related to each other since the characteristics that are advantageous for the competition within sex can be preferred also by the opposite sex. According to both the 'sexy-son' and the 'good genes' theory these traits may be preferred by the opposite sex, since they can be good indicators of the quality and guarantee successful offspring. Although in humans culture-independent preferences of mate choice can be observed in both men and among women, we can see the signs of the significance of men-men competition as well. Increased aggression against other men, the rich facial hair (beard, moustache and eyebrows) the deeper fundamental frequency and low formant positions of the sound spectrum all show a strong correlation with testosterone levels; and these traits could have only a minimal advantage otherwise in the everyday life (e.g. during hunting) (Puts, 2010).

During mate choice a good indicator of genetic quality can be the resistance shown against pathogens, the body symmetry, as well as androgen-dependent characteristics. All these can have perceptible cues that can be used in mate choice. An important inherited feature of the immune system may also play an important role in mate choice, called major histocompatibility complex (MHC). These are genetic regions that show great diversity allowing more effective defence against a wide variety of pathogens. Thus, during the mate choice may be beneficial to find a partner, which shows differences in the largest possible number of different MHC alleles compared to the individual's own (Roberts and Little, 2008). The MHCs appear in body odour that makes it possible to develop a preference, and although not consciously but it can influence the pair selection. This is supported by the finding that there is a significantly greater diversity of MHCs between a man and a woman living in a relationship than the diversity of MHC alleles in randomly selected pairs (Chaix et al., 2008). Characteristics influenced by the androgen hormones (like body size, musculature) can also give a good picture about the defensive abilities against physical threats, but since these hormones inhibit the immune system, here the bearers of masculine traits have worse defensive ability against pathogens. This discrepancy might be explained by the handicap theory, since individuals with a high level of male sex hormone, only in otherwise good physical condition will be successful survivors despite their inhibited immune system. Finally, the degree of so-called fluctuating asymmetry is associated with the potentially harmful mutations in the genetic background, so preference expressed towards the symmetrical traits also can provide better genetic material to the offspring. Accordingly it can be shown that women prefer more masculine-looking, more symmetric men based on physical appearance as well as body odour and voice.

In the following part we will describe easily measurable physical parameters that are known to evoke preference in the opposite gender in humans.

2.2 Body size

In species where there is a significant size variance in one of the genders, and striking sexual body size dimorphism can be found between the sexes, intrasexual selection will be the first suspect background mechanism that comes to mind. But the larger size in one of the genders can be also the subject of the other sex's preference for body height or weight, which is usually an honest indicator of physical force, or the ability to retain resources (resource-holding potential) (McElligott et al., 2001). Generally speaking, in the sexually dimorphic species where males are large, the preference for larger males is typical among females, but this may vary also depending on the relative size difference between the male and the female. Complicating this issue even more, the males may also exhibit size preference among the females, but not necessarily in the same way. For example, in a poeciliid species (*Brachyrhaphis rhabdophora*) it was demonstrated that females prefer larger males over the smaller ones, but the males prefer females with stature like themselves (Basolo, 2004). In the sand lizard (*Lacerta agilis*) there is no detectable preference in the case of females, while males tend to choose to mate with larger females. In general, larger females' fertility is also higher, thus clear preference for larger female body size may increase their reproductive success (Olsson, 1993).

2.2.1 Female preference for taller men

In the case of humans the relationship between body size and reproductive success is proven (Pawlowski et al., 2000), although there may be cultural and environmental differences, which can greatly affect this (Salska et al., 2008). Western societies are characterized by that the taller men are more successful, they reach higher socioeconomic status, and they have more offspring (Pawlowski et al., 2000) (though not necessarily from the same mother: Mueller & Mazur (2001)) The reason for this can be the preference in women for taller men, or, with the same effect, if shorter males face disadvantages during mate choice. This is confirmed by the observation that women show a strong preference for higher men around ovulation, but specifically for short-term relationship (outside their long term relationship) (Pawlowski and Jasienska, 2005).

Interestingly in men only relative preference can be observed: i.e. they looking for shorter partners than themselves (Swami et al., 2008). Although in some cultures there is evidence of a positive correlation between female height and fitness, the taller men here also more likely to marry in general(but of course this comes not with greater number of children necessarily) (Sear, 2006). The fact that men pay less attention to the female body height is not surprising considering that it is less related to the reproductive potential, in fact there can be a trade-off between the two because the extreme heights can come with serious health problems (Nettle, 2002).

2.2 Preferred body ratios

It is often observed that not the body size itself serves as primary indicator of quality for the opposite sex, but rather the size of certain body parts, or the ratio between particular body parts may function as the quality indicator. Specific quality indicators that evolve due to the other sex's preferences over the sexual selection are called ornaments. Typically, this is true to the swordtail fish's (*Xiphophorus* spp.) eponymous characteristic. Some of the lower radial elements of the tail fin stretches in the males, and it forms the so-called sword. The longer is the sword, the more preferred will be the male among the females (Basolo, 1990a). Interestingly, however, the female preference appeared earlier in evolution than the sword extension itself. In the close relatives of the swordtail fish this feature does not appear, which indicates that being swordless is the ancient state, while the females of a sword free species the platy (*X. maculatus*) show preference towards males with artificial sword protrusion (Basolo, 1990b). Thus, being sworldess and having preference for swords together is still more parsimonius explanation than assuming that sword was lost in one point of evolution, and later appeared again due to a new mutation, while the female preference remained intact.

2.2.1 Body ratio preferences in humans

In humans, in addition to many other features, body shape can have a prominent role in mate choice. Both genders show preference to certain typical body proportions, to some extent independently of the cultural traditions of the given population. In women, waist-to-hip ratio (next to breast size), is a major influence on men's mate choice

(Singh and Young, 1995). According to several studies, women with the waist-hip ratio of about 0.7 are the most attractive for men, and most desirable for long-term relationship as well. Of course, it can be also affected by cultural and environmental impacts (Wetsman and Marlowe, 1999; Pettijohn and Jungeberg, 2004). The importance of this feature may become clear when we consider that on one hand the broad hips anatomically facilitate childbirth, and on the other hand most of the fat reserves of the female body stored on the hips and gluteal depots, thus waist-to-hip ratio can be a good indicator of the nutrition status. Moreover, the women-specific waist-hip ratio develops during puberty and disappears after the menopause, providing also a good indication of the fertility status (Henss, 2000).

In men there is no significant difference between the waist and hip circumference, however the width of the shoulders, and the mass of the upper body muscles can be significant indicators of quality. Accordingly, the mate choices of women can be affected by the waist-shoulder ratio. Women have been shown to appreciate more and finding more attractive the mesomorph men with waist to shoulder ratio around 0.6 (Dixson et al., 2003), and potential partners with lower ratios are valued as more suitable only for short term relationships (Braun and Bryan, 2006). The reason probably lies in the fact that (similarly to the body height) a wider shoulder, stronger upper body is associated with higher level of male sex hormones, which is a good indicator of higher competitive ability and good genes, but increases the likelihood of desertion and aggression, so it makes these men less suitable for long relationship.

2.3 Voice as sexual character

Due to anatomical characteristics of the vocal apparatus, many of the perceived acoustical parameters of vocalizations can potentially carry information about the caller's quality and unique features (Taylor and Reby, 2010). On one hand, the characteristics of the fundamental frequency of the vocalizations are mainly depending on the morphology of the larynx and vocal cords, which are influenced by sex hormones during the development. Therefore fundamental frequency (the pitch of the voice) can be a good indicator of the male quality, competitive ability and indirectly his dominance status (Vannoni and McElligott, 2008). In addition, the synchronized operation of the vocal cords is necessary during normal phonation, so any significant asymmetry that appeared during development will cause perceptible irregularities in the sound. Due to the fluctuating asymmetry the level of the noise component in the fundamental frequency can inform potential partners about the genetic quality of the caller (Fitch et al., 2002; Hughes, 2002). On the other hand, since the vocal tract length is usually closely related to body size, the distribution of the formants in the spectrum may serve as an indicator of the body size, which is an important indicator of the quality of the individual (see also Chapter 9). In red deer (*Cervus alephus*) it is well studied that during the breeding season other stags perceive size information carried by the roars and use it to decide whether it is worth to fight and to challenge the roaring stag (Reby et al., 2005). The deer hinds have also a strong open ended preference towards larger sounding stags (Charlton et al., 2007). Third, the emission volume, length, and how long the individual can continuously and persistently vocalize can also be good indicators of the physical state and of the dominance status of the individual (Kitchen et al., 2003; McComb, 1991).

2.4 Preference for acoustical parameters

The importance of certain voice parameters during mate choice was also shown in humans. Men prefer the parameters of the female voice that primarily can be associated with younger age: they find attractive the higher pitched sound containing wider formant dispersion (Figure 1), and these women were valued to be more attractive on the basis of their images only without sound, too (Collins, 2003). Furthermore, it appears that the woman's menstrual cycle also affects men's judgment. The same woman's voice is preferred more if she is around ovulation, i.e. she is ready for fertilization (Pipitone and Gallup Jr., 2008; Nathan Pipitone and Gallup, 2012). Both oestrogen as well as progesterone affect the cells of the mucous membranes in females, and through this the physical properties of the vocal cords may be altered, thus affecting the production of sound that can be detected as an increase of the fundamental frequency (Bryant and Haselton, 2009).

Female preference, not surprisingly, is directed towards the characteristics of the male voice, which are influenced by male sex hormones. Higher levels of testosterone cause primarily deeper fundamental frequency, and has little effect on the position of the formants (Evans et al., 2008). However, the latter may have also an effect on the mate choice of women (Figure 1). As the development of the adult voice due to the laryngeal lowering is triggered by the rise in testosterone levels during puberty, the more lower position of formants indicate sexual maturity. Male voices that have deeper pitch and lower formants are belonging to more masculine, more dominant, larger stature

and older men (Feinberg et al., 2005), and women also find them more attractive (Hughes et al., 2004; Re et al., 2012). In addition, mature men with a voice having deeper fundamental frequency are actually shown to have higher reproductive success (Apicella et al., 2007). However, the influence of female sex hormones can be detected here, too. As we discussed it previously, women in their ovulation period show a stronger preference for traits that can be connected to higher testosterone levels: find a deeper pitched male voice more attractive, but fitting only for short-term relationship (Puts, 2005; Hughes et al., 2004).

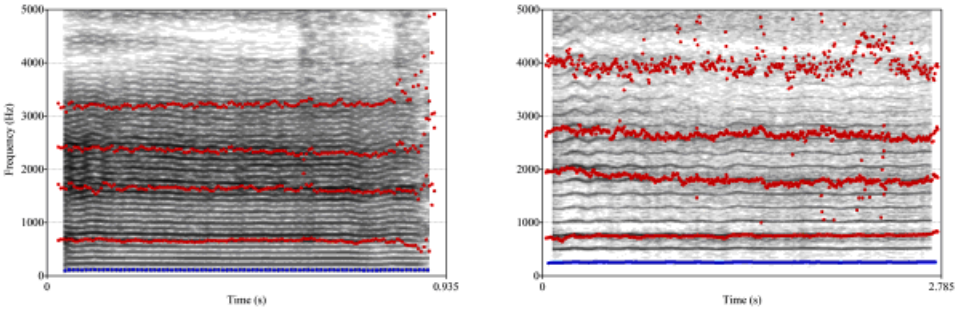

Figure X.1 Sound parameters which are important in the male and female mate choice: the fundamental frequency (blue) and the first four formant frequencies (red). Male voice (left panel) and female (right panel).

3. MATERIALS

3.1 Subjects and equipment

During the study our subjects will be the students who are attending the practical. The voice samples used for sound playback from 15-15 male and female subjects are selected from the sound database collected in the previous years, in a way that they represent the total variance of the population The bodily parameters (height, shoulders, waist, hip circumference, 2D4D length, fundamental frequency, formant dispersion) of the individual callers are known and we will investigate how do they affect the evaluation of voices. Questionnaires will be used for collecting the answers. Data collection is done on paper, the data analysis is performed on computer, with Excel and Instat software.

4. PROCEDURE

4.1 Aim of the study:

During the practical, we study male and female preference based on sounds. The primary question of whether the acoustic parameters of the voice affect how men and women evaluate attractiveness through the human voice, whether this shows any correlation with the physical characteristics of the caller.

4.2 Steps of the study

We collect scale variables obtained of subjective assessment from students in the practical. Scales of 0 to 10 will be used for assessing how attractive the students find each sound, and we will use masculinity-femininity, and possibly dominance scales well. We record the evaluators' gender and age. In addition, it is also possible that some variables suggested by the students will be added to the questionnaires.

The playback procedure is done by the demonstrator, as well as the summarization of the results from the questionnaires. This will be edited together with the acoustic parameters of the sounds and the data of the callers, as well as previous years' survey data, and then released to the students who calculate the data for the statistical analysis independently. During the statistical analysis we compare the responses given by men and women on the group level, and also calculate the assessments for each sound for linear regression to examine how the responses were influenced by various parameters of the callers.

4.3 Preparation of the study notes

Each student prepares the study note individually, based on the analysis and evaluation of the results of the released data from Excel spread sheets.

The record shall include the following details:

- Steps of an scientific investigation.
- Completed copy of the Datasheet with filled letterhead (of course, the data series does not need to be attached)
- Results:
- A short introduction in which the theoretical background of the study is summarized
- Questions and Hypotheses
- Method, description of the measured variables and measurement procedures
- A summary of the comparisons (which statistical method was used and why)
- Comparison of the two groups for all measured variables (six comparison)
- Statistical results (numerically, t and p-values and degrees of freedom are shown; and in the text they should also be explained with one or two sentences)
- Graphical representation of the Results (Excel graph)
- Analysis of the relationship between some variables within groups (three variable pairs - preferably biologically plausible, and relevant for answering our questions - chosen in both groups. Six comparisons.).
- Results (p and r values) in short, full sentences
- Graphical representation of the Results (also Excel, scatter plots with trend lines)
- discussion of the Results up to one page (Do the results match to our hypothesis, if the do not, then why not, etc), biological background, possible explanations for the phenomenon, the possibility for further studies.

The notes' preferred form is made with text editor, printed, stapled as a manuscript, but neat hand-drawn/written version is also acceptable.

Sample Questionnaire

The subject's

Gender: female / male

Sexual orientation (optional):…….

Age: …..……

To what extent is it true for the heard sound that

Not at all very much

Sample1:

a) Attractive 0 --- 1 --- 2 --- 3 --- 4 --- 5 --- 6 --- 7 --- 8 --- 9 --- 10

b) Dominant 0 --- 1 --- 2 --- 3 --- 4 --- 5 --- 6 --- 7 --- 8 --- 9 --- 10

feminine masculine

0 --- 1 --- 2 --- 3 --- 4 --- 5 --- 6 --- 7 --- 8 --- 9 --- 10

Sample2:

a) Attractive 0 --- 1 --- 2 --- 3 --- 4 --- 5 --- 6 --- 7 --- 8 --- 9 --- 10

b) Dominant 0 --- 1 --- 2 --- 3 --- 4 --- 5 --- 6 --- 7 --- 8 --- 9 --- 10

feminine masculine

0 --- 1 --- 2 --- 3 --- 4 --- 5 --- 6 --- 7 --- 8 --- 9 --- 10

Sample3:

a) Attractive 0 --- 1 --- 2 --- 3 --- 4 --- 5 --- 6 --- 7 --- 8 --- 9 --- 10

b) Dominant 0 --- 1 --- 2 --- 3 --- 4 --- 5 --- 6 --- 7 --- 8 --- 9 --- 10

feminine masculine

0 --- 1 --- 2 --- 3 --- 4 --- 5 --- 6 --- 7 --- 8 --- 9 --- 10

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Chapter XI. How intrauterine development affects later rank and anogenital distance in rabbits

Vilmos Altbäcker
Oxána Bánszegi

1. OBJECTIVES

This practical is designed to provide experience for the students in experimental work with living animals. To show them how to measure an animal's – in this case a rabbit's - morphological variables or testing their behaviour; to offer them an opportunity for planning and executing a real experiment, and evaluating the data at the end. Beyond the theoretical background the students learn about one of the important features of the endocrine system: the prenatal effects of the hormones and their long term consequences for the entire life of the animal. During the practical, students will observe and evaluate the social hierarchy, based on the animals' different morphology and behaviour. As of the behavioral tests, the students learn the rules of data collection, and after that the appropriate data analysis and evaluation. They will process the data by the INSTAT statistical software, and at the end of the practical they will learn how to create a graphical presentation of the data (Excel).

2. INTRODUCTION

2.1 Hormones and behaviour

The **neuroendocrine system**, which is the ensemble of the nervous system (neuro), and the endocrine glands (endocrine) controls the operation and functions of the body. The nervous and endocrine systems cannot be separated clearly from each other. The endocrine system is under the control of the nervous system, and the hormones produced by the endocrine glands are acting as signalling material (neurotransmitter, e.g. Epinephrine) of the nervous system. **The endocrine system is a network of glands, each of which secretes their products (called as hormones) directly into the bloodstream**. Even small amounts of hormones are able to elicit their the tissue-specific function, and influence the metabolism at distant parts of the body. The hormones have **organizational and activation effects** as well. They affect the development of the tissues via the organizational effect, and doing so their impact can be long-term, or even irreversible. Hormones remain operational also in the adult organs via their activation effects.

2.2 Prenatal hormonal effects

In litter bearing mammals the same-sex individuals within the same litter may differ from each other in their morphology and behaviour, but these differences are not only due the genetic factors . A number of different effects apply to the embryo at **prenatally** through the placenta. Probably the most important are the hormones, as they greatly influence the subsequent embryo morphology and behavior. The **hormonal imprinting** is when a small amount of hormone at the right time induces long-term and irreversible changes. In the **sexual differentiation** the most important factors are the sexual hormones, and among these the testosterone has a special role.

The gender is primarily determined at fertilization by the genetic setup of the zygote; however the male or female morphology of the individual only is only formed during the fetal development based on the process of sexual differentiation. The sexual differentiation depends on hormones that affect the fetus' male sex characteristics known as **androgens**. From the initial state of the brain, the development moves towards male characteristics. In mammals, the product of one of the genes on the Y chromosome is essential for the development of the male genital gland. It is a protein which provokes that the trabecular epithelials transform into tubes in the gonads and the testicles start to develop. Androgens and particularly the testosterone, which are produced by the developing testicles, trigger the gender distinction. Androgens influence the further development of the genitalia, and as their impact,

the anti-Müller hormone is produced, which is launching cell death in the Müller-tube. Meanwhile, the Wolf-tubular differentiates into vas deferens, and epididymis. If those do not develop properly in the testicle, the hormones are produced in the Müller tube and the development goes towards a female direction resulting in an oviduct, uterus and vagina.

Testosterone or one of its metabolites influences parts of the central nervous system that are responsible for the subsequent sexual behavior. During their development, male fetuses start to produce testosterone earlier and in larger amounts than females do. Testosterone, which is produced in the male fetuses during a sensitive period of their intrauterine development, can affect the brain's sexual differentiation. Testosterone production reaches its peak at the time of the sexual differentiation. . The proper amount of testosterone in a developing male foetus is essential, and it cause long-term effects.

In mammals, testosterone can easily penetrate the foetal membrane therefore the position of a particular foetus relative to its siblings affects its chances to be reached by the hormone produced by its male neighbours. As a consequence, **intrauterine position** (IUP) affects a variety of morphological and behavioral traits in mammals (including humans) giving birth to several offspring at the same time . As per the 'contiguous male hypothesis', testosterone affects the direct neighbours of the foetus, diffusing through the amniotic fluid and the foetal membrane. In utero, female mice having 2 male adjacent littermates (2M female) are exposed to larger amount of testosterone and became less feminine and more masculine than those having one or no adjacent males (1M and 0M females) .

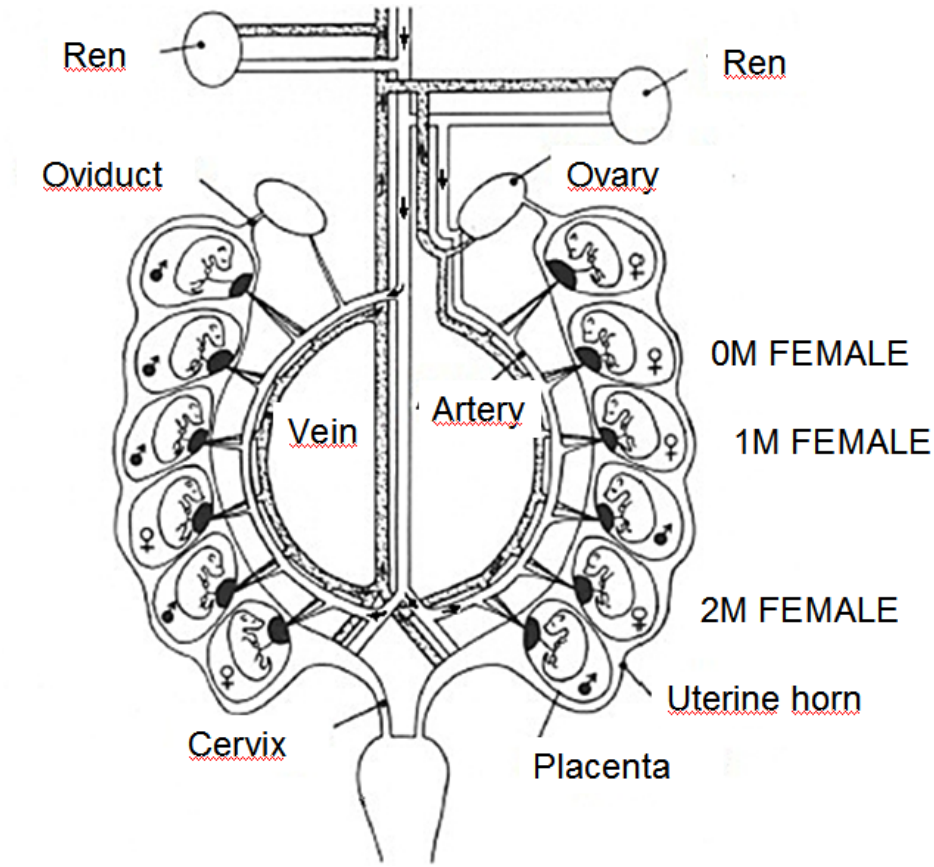

Figure XI.1 *Schematic diagram of the uterine horns and uterine loop artery and vein of a pregnant mouse. Arrows within the loop artery and indicate the direction of blood flow. Blood flows in the uterine loop artery in a caudal direction at the point where the fetus was implanted at the ovarian end of the uterus and in a rostral direction where the foetus was implanted at the cervical end of the uterus.*

Thus, at least part of the behavioural variation in mammals may originate from differences in foetal physiology: the **male foetuses in mammals have a higher blood plasma level of testosterone than females**. Comparing serum testosterone levels of females of different IUP revealed significant differences in Mongolian gerbil and house mouse foetuses, as testosterone levels depend on the number male neighbours. The **2M-female foetuses**

had higher concentrations of testosterone in the blood and in amniotic fluid, than 0M foetuses and this difference was still present at adulthood.

The difference in sensitivity to testosterone can be found in a number of species between 0M and 2M and females. Those females (house mice and rats), which were exposed to extra testosterone at embryonic age - from neighbouring male embryos or externally - show much faster and more intense response to testosterone treatment after puberty. They become also more aggressive, initiate fights more often.

Intrauterine position of an animal often affects certain parts of the central nervous system, which might may affect behaviour in many forms. As a result of extra prenatal testosterone, mating patterns of typical female's behaviour (e.g., lordosis position) are reduced, and the typical male's behaviour, such as trying to mate with other females increase. Females became less feminine by as the effect of testosterone, as 2M females showed vaginal opening at a later age and gave birth to fewer litters than 0M females during their lifetimes, and the sex ratio was male biased in these litters (Clark, Karpiuk, & Galef, 1993). The 0M house mouse females are more attractive for males. This attraction is partly based on odours. Marking the area with urine and secretions gonads glands is a form of aggression in mice, and this behaviour is a characteristic of males, and therefore more typical in 2M females than 0M females. Males are much more attracted to the smell of 0M females than 2M females (Drickamer, Robinson, & Mossman, 2001). 2M females are more aggressive than 0M siblings during pregnancy and lactation. This difference may be related to lower anxiety levels, as 2M females show less combat evasion than 0M females.

Examining the birth weight in mice, there was no difference between the sexes. There is no difference within the sexes depending on their intrauterine position either. However, later in the development in both sexes, there will be a weight difference between and within the sexes. The males are heavier than females and in both sexes 2M females are heavier than the 0Ms .

AGD as a biomarker

In many mammal species, some **sexual differentiation in the morphology** can be observed even at birth at least at the genital region. **Anogenital distance (AGD, distance between the anus and the genitalia)** exhibits sex related variation in certain rodent species (and also in humans) indicating that AGD is a reliable indicator of prenatal androgen exposure in sexual differentiation. As known from studies conducted on mouse, the **AGD depends on the IUP, as 2M females have longer AGD than 0M females**, while 1M females are intermediate .

The California mouse (*Peromyscus californicus*) show similar trend to other rodents in the morphology of the anogenital region, however until weaning the AGD of the two sexes is overlapping, and only after that the difference appears. However both at birth and in adulthood, females from a male biased litter with sex ratio larger than 75% had a larger AGD.

Several prenatal androgen-treatment experiments showed that the testosterone has a dose-dependent effect on the anogenital distance. However, prenatal anti-androgen (flutamide, cyproterone acetate) treatment negates the effect of male neighbours in utero. Consequently, over the past few decades, AGD become a widely accepted biomarker and used in the prenatal testosterone-effect studies.

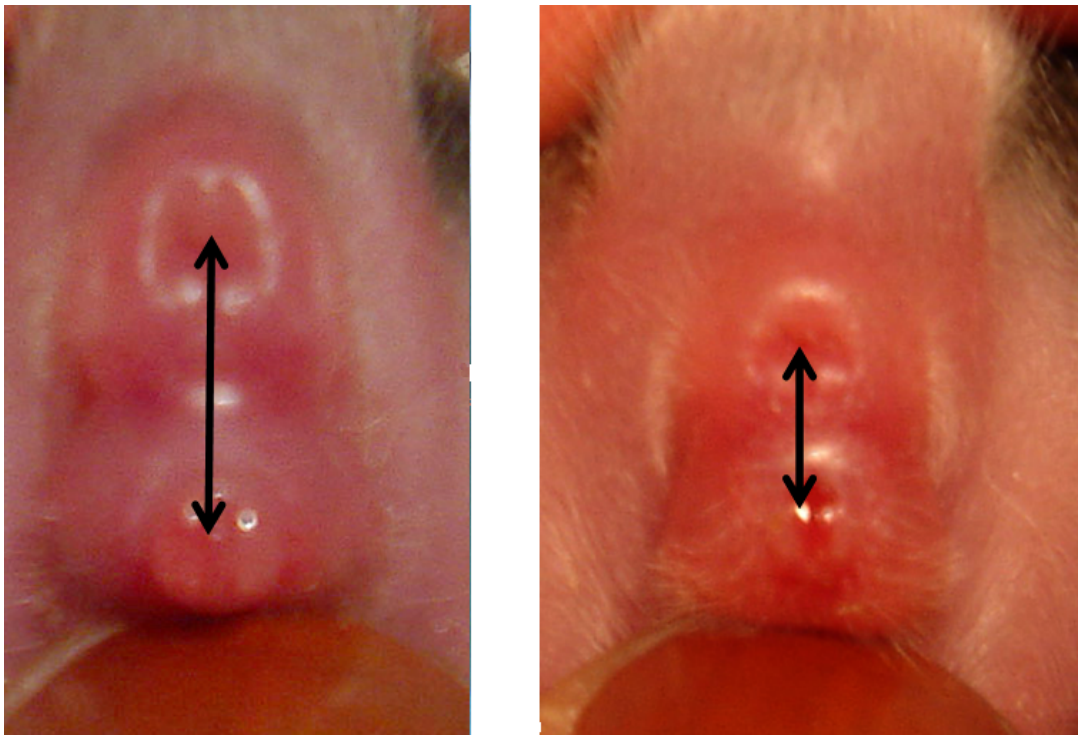

Figure XI.2. anogenital distance of a male (left) and female (right) newborn rabbit. Anogenital distance difference among the male and female rabbit pups is already evident at birth.

Sexual differentiation in rabbits

In rabbits, the foetal gonads begin to differentiate at day 14 of the fetal foetal life. From that time the level of testosterone of the male gonads will be ten times higher than females', proving that there is endocrine activity in the foetal testes. The testosterone level in male pups reaches the maximum on day 20-21 after birth, which is the critical time of sexual differentiation. Different tissues of reproductive organs in both sexes show sensitivity to testosterone from day 18 in utero.

Rabbits - although being not a rodent – are frequently used experimental animals not only because of the above-mentioned reasons, but there are several other features of rabbits making them appropriate animal models. For example their placenta is of hemochorial type, similarly to guinea pigs, rats, mice or humans. Therefore rabbits are widely used species for testing permeability of the placenta or foetal physiology and development, because the good comparability of the results with the above mentioned species. One of the consequences of how the material chemical compounds flow through the placenta is that the mother's diet affects the foetuses' later food preference (Bilkó, Altbäcker, & Hudson, 1994).

The gender difference in the morphology of the external genitalia can be observed in the rabbits as well. Males have larger anogenital distance than females. There were several studies conducted at the Department of Ethology in the ELTE, researchers conducted a number of experiments bout the rabbits' sexual differentiation. Large individual variation was found in the anogenital distance of adult female rabbits, and this morphological trait has been linked to other behavioural variables. Dombay et al (1997) examined the relationship between anogenital distance and spontaneous chin marking activity. As expected, it was found that females with large anogenital distance showed elevated chin marking activity over females with small anogenital distance. In another study, it was found that rabbit males responded with different over-marking activity showing a stronger response to chin marks of females with small AGD than of females with large AGD. Bánszegi et al (2009) also revealed that AGD in rabbits is a reliable indicator of sex, as male pups had larger AGD than females both at birth and later on. Furthermore the adjacent male foetuses had significant effect: the more adjacent male foetuses females have had the longer AGD they developed. 2M females had the largest AGD at birth and through the adulthood too. AGD at birth was a good predictor of the AGD and behaviour of adults, as 2M does showed the longest AGD and the highest chin marking activity among females. In a second study these researchers revealed that does with large AGD have sig-

nificantly smaller and lighter litters with a male biased sex ratio; with fewer females but not more males in the litters (Bánszegi, Szenczi, Dombay, Bilkó, & Altbäcker, 2012).

Social system of rabbits

Wild rabbits are **gregarious animals**, living in **colonies**. Each colony has a **dominant** and some **subordinate** bucks and five to six females and young animals. The related females live in a large burrow with multiple entrances or several small burrows which are close to each other. Males defend several burrows in their territory. **There is a strict hierarchy among both males and females**, males compete for females, and females compete for better nesting sites. **Reproductive successes of the higher-ranking females are much larger** because they have the safer - more protected from predators or flooding - nesting chambers. Females protect only the burrow and its surroundings against foreign rabbits, males defend the whole territory against foreign males. The home range size of rabbits is not very large, it is usually limited to 20 meter radius zone around the burrow, however, the colony's territory, can be larger than one hectare depending on the population density. The bucks mark their territory boundaries with faecal pellets, and with chin marks.

In case of rabbits, it can be said that from evolutionary reasons, **males should prefer small anogenital distance females**, since those produce more offspring and with a higher female ratio, therefore greater reproductive capacity. **A buck can maximize his reproductive success, if he prefers the small anogenital distance females** and in addition mate with all the large anogenital distance females with which he can. However, females with large anogenital distance are not only more aggressive but also produce smaller litters with more males. Thus, the invested time and energy in a female with large anogenital distance may have a negative impact on reproductive success of a non-selective buck. However, females with large anogenital distance are more aggressive and may became dominant over the other females, and they get the optimal nesting sites that are less exposed to predators and flood risk.

3. MATERIALS

3.1 Experimental animals and methods

The practical takes place at the Biological Research Station of Eötvös University, Göd. We use female wild rabbits. When adequate numbers of animals are not available we will use video recordings of the earlier experiments for the analysis. During the practical the students have to compare the behaviour of females with different anogenital distances in a social interaction test. They establish the hierarchy formed among the females with the analysis of the behavioural variables. During the observation the data collection will be done by pencil on paper, then the data will be analyzed in Excel and Instat software packages.

4. Procedure

4.1 Goal of the practical

1. Measuring anogenital distance and weight on living animals

2. Social interaction test is performed on four animals. We have to select four female rabbits with nearly identical weight but different AGDs. The observation of the animals starts after placing them into the test arena. The goal is to gain experience in handling live animals and coding behavioural variables during direct observation or video recordings.

Behavioural variables:

- eating
- initiate a fight
- chin marking in the arena
- put chin mark on other animal
- crossing the imaginary lines dividing the arena to four quarters

4.2 Steps to be followed:

1. **Measure the weight and anogenital distance of the individuals.**

We place the animals together into the arena. The first 5 minutes is called habituation time, the test starts after this time has passed. The observation lasts 15 minutes during which we note our variables on the test sheet.

During the tests we have to count how many times each animal eats, starts a fight, puts a chin-mark in the arena, or on another animal, or how many times it crosses the partitions of the arena. The data should be summed every 5 minutes. Five successive tests must be performed on different animals. If there is no adequate amount of animals, previously recorded videotapes will be analyzed.

1. type the data to MS Excel

2. Analyze the data, calculate mean and standard deviation

3. Prepare a graph (Columns with means and SD)

4. Choose a statistical method to analyze the data in INSTAT

5. Draw consequences.
   - How concordant are the results with previous findings?
   - If not, how can you explain the results?
   - Ask further questions based on the test/results?

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Chapter XII. Risk taking in animals and humans: gender effects

Vilmos Altbäcker

1. OBJECTIVES

This practical is an introduction to human risk taking behaviour. Risk can be defined as the willingness of individuals to put their life in danger in order to gain benefits. Men are more risk taking than women. In particular, young men are more risk-prone than any other demographic category. This young male syndrome has been documented across a variety of behavioral domains and cultures. Men should compete elaborately in early adolescence, because they have to gain social status, which contributes to their abilities to provision for future offspring. Within the same age class, single men are expected to take more risks than men involved in a relationship. In this practical, we will document if risk taking in hazardous traffic situations is more characteristic to men than women, and if it is age related.

2. INTRODUCTION

Males and females have different preferences and show gender specific behaviours. Tendencies towards certain types of behaviour, including less-safe driving, are 'hard wired' in men. Teenager drivers in particular are famous of their craziness, and when asked they may give a rational explanation, but the biological reason behind their act is that they take risks as an advertisement of their willingness to show their abilities (Kruger and Nesse, 2006). Risk can be measured on two axes, probability and severity, and the combination of these two factors determines how serious a risk is.

Men and women are different in their driving behaviour, the differences can be seen clearly in the enhanced probability of males to take risks and to show aggression in road encounters. The consequence of these differences is very obvious in higher accident statistics worldwide. These differences may be reduced by socialisation, but they are rooted in more fundamental factors. Evolutionary psychology provides a strong basis for sourcing many of these back to the hardly changed cognitive structures required by our hunter-gather ancestors.

Male-biased risk taking can be explained using the theory of parental investment. Males just follow their built in motivation which is present of most mammalian species and appears in maturing males. In humans, this is neither a cultural artifact nor an excuse for suspects of traffic accidents; it is a widespread tendency of biological origin.

It is not at all unusual in animal kingdom that one of the sexes takes much higher risk than the other (Pusey, 1987). At a certain age, members of one sex of a species show a high tendency to start large scale movements, or dispersal. Individuals apparently disperse due to intrinsic factors rather than extrinsic or environmental factors. Although parental aggression may also be involved, the tendency to disperse during a particular period found in several studies suggests that the intrinsic components are more important in the dispersion. In Belding's ground squirrels (*Spermophilus beldingii*) the trigger for dispersal was achieving a certain body size (Holekamp, 1986). A juvenile ground squirrel must reach an optimal body mass before leaving its den. The juvenile will remain near the den until it achieves the body mass and fat reserves which are necessary to survive the next winter. Individual differences in pre-dispersal body mass of the male squirrel correlates with winning fights during the next mating season and hence with mating success. If a ground squirrel left the den too early, even if he survived, he may not be strong enough to win fights and mate next year.

Another intrinsic reason for dispersal is related to individual development. All animals go through certain stages of development, including infancy, puberty, and adulthood. Studies of mice (*Mus musculus*) show a marked increase in their motivation to explore at puberty (Macri, et al., 2002). Adolescent mice have a reduced basal level of anxiety during puberty exhibiting a high level of risk-taking behaviors. Since exploration of unfamiliar environments is often associated with anxiety, mice often disperse at this time. Marques et al., (2008) also found that exploration and risk taking behaviour are critical to enable pre weanling mice to cope with novel situations and gain control over their environment after the dispersal.

Long distance movement through unknown environments increases mortality and its energetic cost is high, so it is generally considered as a risk-prone behaviour. Adolescent men are in a transitional life period as they start to disengage from their family trying to attain independence. They often do it through risky actions, hence often characterized as risk-takers as they perform an array of risky behaviours. Adolescent men also push the social limits by seeking extremities like casual sex, smoking, gambling.

Natal dispersal occurs in virtually all birds and animals (Dobson & Jones. 1985), and when birds and mammals are compared, some striking trends emerge. Greenwood (1980) examined studies representing 30 different species of birds and mammals and found that the number of species of birds and mammals where natal dispersal is more extensive in males or females shows opposite relationships. He has reached three conclusions. 1). In both birds and mammals, one sex usually disperses more than the other. 2). In birds, females disperse more than males. The male bird defends a territory and females may choose a male on the basis of his territory quality. It may pay a male to remain near his birth site because it might be easier to set up a territory in the vicinity of relatives. Once this happens, it may be adaptive for the females to disperse to avoid inbreeding. 3). In mammals, males disperse more than females. A male will benefit most from gaining access to a large number of females and so male dispersal may have been favored (Greenwood 1980).

It is often assumed that risk-taking behavior increases the chances of a male to reproduce. Of the males who take large risks, some of them are unlucky and die without any children, but others are luckier and produce large numbers of children: boys carrying the genes for male risk-taking, and girls who prefer males who take risks. Consequently, both the frequency of genes for risk-taking in males and female preference for it could have increased in mammalian species. This is the course of natural selection, supporting the predictions derived from the theory of costly signaling (Farthing, 2007). Women would prefer takers of non-heroic, as well as heroic, physical risks as mates over risk avoiders, provided that the risk level to the potential mate is low to moderate. Men who take low to moderate non-heroic physical risks may be successfully signaling desirable traits such as bravery, compared to risk avoiders. But those men who take high level risks should not be preferred as they could be seriously injured, thus reducing their value as mates. For heroic risks, on the other hand, the altruistic component of the risky acts is such an important signal of mate quality that it can overcome worries about risks to the mate's physical safety, such that heroic risk takers may be attractive as mates even when the risk level is very high.

3. MATERIALS AND METHODS

We will study gender differences in human risk taking behaviour, namely traffic rule acceptance. Instead of data mining from National Databases for statistics of traffic accidents by sex, we will actually monitor how people follow rules in risky situations like traffic lamp crossing. Based on above considerations this is a non-heroic medium risk situation where we expect gender difference in rule breaking.

The practice starts by answering short questions on the gender difference in behaviour in the seminar room, and then we visit the nearest tram stop to obtain data. Coming back we analyse the data and finish by filling the discussion points given below. You must submit the one page report and the ORIGINAL data sheet.

Depending on when the practical is scheduled, we collect data in one of two places. In the morning, we gather data at the tram stop next to Petőfi Híd. The afternoon alternative, which is also convenient in bad weather or in periods when traffic is low at the University, is the tram stop at the Nyugati Railway Station. In both places, pedestrians are not allowed to cross the three lanes except at the traffic lamp, which is placed at a rather inconvenient position. Other legal option is using the stairs. Nevertheless, you will observe people crossing the road among cars running at 60 km per hours.

Our initial impression (obtained by simply observing trams without recording data years ago, and documented during the same practical held in the last year) is that rule breakers are mostly men, but such an outcome might stem from several reasons as possible explanations:

1. it was a temporal fluctuation, there is an even sex ratio of the rule breakers on a long run. (NO DIFFERENCE)

2. there are more men than women coming by tram to this stop, and their further behaviour is simply mirroring their proportional distribution (DIFFERENCE DUE TO BIAS IN TRAVELLERS' SEX RATIO)

3. there is a real bias in breaking the rules (GENDER DIFFERENCE IN RULE BREAKING)

We will try to exclude some of the above explanations by gathering empirical data. Thus, we will monitor if gender differences exist in risk taking in a hazardous traffic situation, or gender of rule breakers fluctuates by chance, just because the composition of travelers also fluctuates. We need a series of similar events as samples to decide which explanation is against the real world data. As events, we will observe people leaving trams and crossing the main road. Good observation points are the southern roadside of the Buda tram station at Petőfi híd, or the stairs of the Nyugati railway station. Our plan is to collect full sets of data from at least 10 such events (labeled by the exact arrival of trams). Other important aspects of data collection (time window, etc.) will be decided at the tram station after the initial impressions are formed during the arrival of three trams.

By observing the arrival of trams, we decide to collect data on the

- Number of men leaving the tram (offM)
- Number of women leaving the tram, (offF)
- Number of men crossing the road (crossM)
- Number of women crossing the road (crossF)
- Optional, for certain students:
- Number of men using the stairs (stairM)
- Number of women using the stairs (stairF)

Then we calculate the:

- sex ratio of people arriving to the tram stop as the Ratio of men leaving the tram (offRatio)
- and relate it to the Ratio of men crossing the road (crossRatio)
- sex ratio of people following the legal option (stairs): Ratio of men using the stairs (stairsRatio)

In order to calculate proportions accurately, it does matter what is the minimum number of people in an event, therefore a minimum of 5 people should cross the road, otherwise the event is disclosed (see sample data sheet from last year, Fig 12.1 below).

Data will be analysed after the full set of data is collected and we return to the seminar room.

As the ratio of rule-breakers is dependent on the ratio at the arrival on that particular tram, data are not independent. Therefore we will compare our data with PAIRED T TEST built into INSTAT 3 program. Please download the program and read its howto doc from the homepage http:\etologia.aitia.ai. Data from last year are contained in file named kockazathn.xls. You will obtain a Data sheet at the beginning of practice.

Coming back from the tram stop you should analyse the data, calculate sex ratios, compare them with paired t tests, and answer to the following Discussion points at the bottom of the page:

Which gender takes more risk?

Did you notice age dependence of the rule breaking?

What are your suggestions on how should the study proceed?

Fig 12.1 is the data sheet to be used. It also shows data from three events in the previous year.

Figure XII.1 Data sheet for the practice of human risk taking

DATA SHEET .....FOR THE .HUMAN RISK TAKING PRACTICE

DATE: **EXPERIMENTER:**

TRAM No	offM	offF	crossM	crossF	offRatio	crossRatio	Remark
1...b1221	8	11	2	1	0.42	0.67	too few data
2...b2178	5	6	4	3	0.45	0.57	.....
3...b1127	9	6	5	1	0.60	0.83	.....
1.....	.....	.....	...	....	.....	.....	.....
2.....	.....	.....	...	....	.....	.....	.....
3.....	.....	.....	...	....	.....	.....	.....
4.....	.....	.....	...	....	.....	.....	.....
5.....	.....	.....	...	....	.....	.....	.....
6.....	.....	.....	...	....	.....	.....	.....
7.....	.....	.....	...	....	.....	.....	.....
8.....	.....	.....	...	....	.....	.....	.....
9.....	.....	.....	...	....	.....	.....	.....
10.....	.....	.....	...	....	.....	.....	.....
Means:	.....	.....	....	.....	.....	.....	.....
STDEV:	.....	.....	....	.....	.....	.....	.....

STATISTICS: t(....)=..... t(....)=.....

(offRatio versus crossRatio)

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Chapter XIII. Huddling behaviour in mice

Vilmos Altbäcker
Zita Groó

1. OBJECTIVES

During this practical the students will be able to get a theoretical insight to the basic ethological mechanisms of group formation. After designing the experimental protocol on their own, the students will observe the influence of kinship on a cooperative behavior, the huddling. Mice serve often as experimental subjects for all for all sort of biological studies, since they are easy to keep, and they have a sort generation time. By now, the behavior of house mice strains used in laboratory studies has been diverged from its wild living relatives. During the practical the students can observe the behavior of a wild living native mouse species (either the house mouse or the mound building mouse), and additionally they will be able to learn how to handle these animals, and how to determine their sex.

In the course of the practical the students will perform a complete ethological study, during which they will be able to get familiar with the steps of an ethological survey, as well as with the designing and conducting a scientific experiment.

2. INTRODUCTION

2.1 Animal groups

Individuals of most animal species are often to be found with other conspecifics. These groups can be **temporary** or **permanent**.

Group living has numerous advantages for group members: enhanced protection against predators, more effective foraging, optimal use of resources, easier access to mates, possibilities for communal nursing, increased protection of young individuals. Grouping may also provide thermal benefits for participating individuals by reducing heat loss in cold temperatures if animals huddle together.

Group living has its costs as well: increased competition for the resources, increased probability of transmitting diseases, disturbing each other in reproduction or even killing of the others' offspring; increased chance of the negative effects of inbreeding.

2.2 Types of groups

Aggregation: a simple gathering of animals that, is not based on social affiliation, but on such external constrains and factors like limited accessibility of water, following particular migration routes or using common overwintering places, which make the animals congregate and stay together. In such temporary groups, there is no visible social structure, or cooperation, each individual behaves selfishly.

Groups, which are based on social affiliation:

Anonym group: Groups without individual bond. These can be open or closed.

- **open anonym group**:

In an open anonym group the individuals can join and leave the group freely like it was observed certain fish species. Another type is when smaller groups with individual bonds are aggregating together in a bigger group, like birds nesting in colonies.

- **closed anonym group**

In the case of a closed anonym group, there is no connection between the individuals, but group members recognize each other by a common sign, and they behave peacefully only towards to the group members. Conspecifics that are not wearing the common identifying cue ar signal are being chased away from the group. Mice and rats belong to this kind of group.

Individualized group: In the individualized groups the group members recognize each other mutually. In general the individuals make social ranking, by establishing a hierarchal structure. Vertebrate species with developed social structure (monkeys, particular species of the Canidae, lions) live in these kinds of groups.

Groups can be categorized also according to their function (Brown, 1975):

a/ Kin groups:

In a kin groups the group members are more or less closely related to each other.

- **colonial groups**

In colonial group type, all individual has the same genetic structure

- **family group**:

In case of a family group one or both parents live together with the youngest of its offspring.

- **extended family**: In case of a big family, the offspring which are not able to reproduce on their own yet are are staying with their parents and younger siblings as well.

b/ Breeding groups

- **monogamous pair:** In this case the bond of the given couple lasts for a lifetime, like certain gibbon species, or in birds like the greylag goose (*Anser anser*)**.**
- **harem:** A single male defends more females (many times it can be over a dozen of them), and there is no co-operation between the females in general. Such groups can be found in the hamadryas baboons (*Papio hamadryas*) and red deers *(Cervus elaphus)*.
- **lek:** In this case the males are held together by their affiliation for the mating place. On a certain location, males establish at first the social hierarchy among themselves, and the later arriving females mate almost exclusively with the high-ranking males that are in central positions. Typical lekking species are the American wild turkey (*Meleagris gallopavo*) and the great bustard (*Otis tarda*), a native species to Hungary.
- **spawning group**:

It consists of several males and females, and it is most common among fish species, although it can occur in amphibians as well. This type of short-living group exists exclusively during the time of spawning

- **colony:**

Colony occurs in general among birds, consisting of a nesting pair and other a smaller harems.

c/ Permanent groups

they are present outside of the mating period, between non-kin individuals, and based on the social affiliation between certain individuals (like foraging groups).

The four components of inner regulatory factors which that determine social behavior, and have an effect on every social behavior in some extent:

1. social affiliation

2. ability to communicate

3. aggression

4. social function

Social affiliation: Social activity can happen only when individuals search each other's company.

Recognition of conspecifics: We talk about true recognition of conspecifics when in the presence of conspecifics an individual's behaviour changes specifically (or in other cases the individual shows specific behavior in the presence of a conspecific). Recognition can be based on external cues like body coloration, chemical substances, pheromones, or the mating calls of males. Some animals are able to discriminate not only their conspecifics, but their subspecies too. Conspecifics recognition has an important role in avoiding hybridization, which often causes unviable or sterile offspring.

Kin recognition:

In many cases it is important that animals recognize not only their conspecifics but their kin as well. Relatives carry more or less the same gene variants, therefore it can be disadvantageous to breed with them (because of the negative effects of inbreeding), but sometimes they are worth to cooperate with.

Kin recognition in mice has **two components**, one is **genetic**, which is based on the recognition of immunogen complex, which in turn results in odor preference, and the other is **learned**, caused by **early imprinting.**

Altruism:

Altruism is the behavior of an individual that raises the reproductive success of another individual, meanwhile seemingly decreasing its own fitness: sharing of food, or emitting alarm calls are some of the better known examplars of altruism.

It seems that altruism is a mechanism that cannot be explained by natural selection. There are several explanations for the evolution of altruism:

-Kin selection (Hamilton1964):

Kin selection is based on the fact that relatives share a larger set of common alleles than non-related individuals. For example full siblings share the same genetic material with 50% chance in average. Fitness of an individual (the extent to that an individual contributes to the gene pool of the next generation), can be amplified not only by increasing the **own reproductive success** but helping relatives who are carrying a greater than average share of the same alleles. As the animals tend to maximalise their **inclusive fitness**, this requires kin recognition.

-Reciprocal altruism (Trivers 1985):

According to reciprocal altruism, an altruist act of an individual will be returned later, therefore its development does not require closely related individuals. However it needs individual recognition, to know who the altruist was.

Cooperation

Cooperation is when individuals achieve something together, which they would not be able to do alone. The co-operation requires extra costs from the cooperating individuals, hence it can only exist, according to the evolutionary theory, if it can increase the fitness of the individuals, which can be achieved directly by the cooperative behavior, or indirectly by spreading of the cooperating individuals' alleles through their offspring (Axelrod & Hamilton 1981). Cooperation through the spread of the gens is the result of kin selection (Hamilton 1964) mentioned above. The cooperation of non-kin individuals is usually explained by some form of reciprocal altruism (Axelrod & Hamilton 1981; Trivers 1971).

2.3 The cooperative mound- building mice

The mound building mouse (*Mus spicilegus*) was originally described byPetényi Salamon in the area of Felsőbesnyő, Hungary, in 1882. The mound- building mouse is the only mammal described by a Hungarian scientist, but for long time it was considered to be a subspecies of the house mouse, so the older descriptions of its ecology are mixed with house mouse. It has been considered to be as a separate species only since the 1980s (Orsini et al. 1983). The mound building mouse looks very similar to the house mouse, but its fur color is homogenous grey,

without the reddish hue. The tail is thinner and shorter than the in house mouse, the belly is white with a sharp border line separating it from the back's color, and the front paws are white as well. The mound building mouse has interesting features from several aspects (Bihari 2004). It does not go to the human settlements for overwintering like the house mouse, (Carlsen 1993), so it is more exposed to harsh environmental conditions in the cold season.

With a cooperative effort several mound-building mice (*Mus spicilegus*) build **communal mounds** from soil and plant material **during the autumn**, and they spend the winter together under this construction. The mound does not serve as a food storage as it was believed for a long time, but its function is the protection against moisture and reducing temperature variation of the soil above the nest (Szenczi et al. 2011). Their nest is approximately 90 cm deep in the ground under the mound, where the mice overwinter communally (Sokolov et al. 1998) . The mound has a complex layered structure. The average diameter of a mound is 1.5 m, and it is approximately 30 cm high - it is a very large construction compared to the size of the mouse. It is made by several individuals, by collecting a considerable amount of plant ears (approx. 50 l) and piling up a lot of soil (approx. 200 l). This behavior is unique among the mouse species. For communal overwintering **decreased aggression** is necessary among the individuals, which is possible because of the **blocked maturation of the individuals living in groups** (Feron et al 2003), until they leave the mound and start to reproduce. Therefore their dispersion occurs relatively late, compared to other mouse species. The age of dispersal is 6 month of age in the mound-building mouse, as it was also shown in laboratory studies (Groó et al. 2013). In Hungary the presence of mound-building mice is linked to agricultural fields, but its plant use is restricted to weeds and grasses so they are not considered as pests of crops (Bihari 2004). Mound-building mice use monodicotyledonous plants for nest building, however they use dicotyledonous food and as a plant fill in the mound (Szenczi et al 2011).

2.4 Conspecific and kin recognition in mice

Rodents recognize their conspecifics, and gain information about their social environment by olfactory cues (Brown 1979). This information can be about species, age, gender, social status, reproductive status, group membership, familiarity or individual traits (Gheusi et al. 1996). The recognition of conspecifics is very important in reproduction to avoid fitness decrease caused by hybridization. The house mice are **able to discriminate even between different subspecies based on olfactory cues,** which is very important in sympatric populations (Ganem & Smadja 2002). Social recognition is the ability of sorting the conspecifics to relevant social categories (for example male, dominant, group member, kin, familiar). In this case no previous experience is needed. Individual recognition is based on individual traits and previous knowledge (Zayan 1994). Rats for example are **able to recognize individuals** (Gheusi et al. 1996). Recognition in most cases **based on the odorant substances found in the urine**. The variation of MHC genes determines an individual oudor consisting of volatile carbolic acids which can be detected from urine, even in small quantities (Singer 1997). Main urinary proteins also carry information about genetic relatedness, and they occur in higher concentration than carbolic acids (Hurst 2001).

Kin recognition is the ability of an individual to react towards relatives differently based on the genetic similarities between the two. This phenomenon is considered as common among animal species (Busquet & Baudoin 2004). Avoiding the breeding with close relatives, but helping them at the same time has evolutionary advantage (Hamilton 1964), but this requires kin recognition. **Recognition** can be **indirect** when it is based on the context and certain conditions, or **direct**, when it based on certain individual traits (Waldman 1988).

House mice also recognize their conspecifics. House mice living in the wild are able to recognize their kin, and they show a preference towards unrelated partners for breeding (Krackow & Matuschank 1991). According to König (1989) those house mice females that raise their pups in a communal nest with close relatives gain bigger reproductive success. In seminatural populations, **house mice** females with **similar histocompatibility** complex prefer to nest together (Manning et al 1995); hence it is likely that kin recognition in house mouse **is based on the similarities in their histocompatibility** complex constitution**.**

Discrimination among particular conspecifics is also present in the mound-building mouse. Male mound-building mice can discriminate between two mound-building mice males, but not between house mouse males (Gouat et al 1998). Male mound-building mice can discriminate not only their brothers, but also their cousins from a non-kin male (Busquet & Baudoin 2004). Mound-building mice females can discriminate their unfamiliar sister (raised isolated) from an unfamiliar, non-relative female (Baudoin et al 2005). The experiments of Colombelli-Negrel and Gouat (2006) have shown that mound-building mice are not only capable of individual recognition but they can detect changes in the diet of the conspecific, and they can handle it separately from the odor cues used for individual recognition.

2.5 Huddling

Huddling is a behavior **often shown by animals to decrease heat loss** (Alberts 1978; Bautista et al. 2003; Boix-Hinzen & Lovegrove 2001), this way they are able to allocate the spared energy to other important processes like growth, reproduction. Huddling behavior therefore increases the benefits of group living, favoring the formation of temporal aggregations. This behavior is extremely important for small rodents that are frequently exposed to heat loss because of their small body size. In unfavourable environments, where food and water is hard to find, animals often decrease their energy expenditure by huddling (Scantlebury et al. 2006). At colder climates surviving the harsh winter period has a crucial importance (Wolff & Lidicker 1981). By huddling, animals can lower their energy expenditure in three ways: by decreasing body surface exposed to the cold environment, by increasing the outside temperature of the environment within the group, and by decreasing their own body temperature as an effect of physiological processes (Gilbert et al. 2010). Even the otherwise solitary animals like wood mice (*Apodemus sylvaticus)* gather together in dense overwintering groups (Wolton 1985), hence thermoregulation dis an important factor in the evolution of group building (Beauchamp 1999). Huddling is a form of cooperation that requires that the individuals (at least temporarily) should not be aggressive towards each other. In mound-building mice the grouping effect suppresses maturation and therefore also the levels of aggression, linked to the onset of maturation.

Huddling can be costly, for example time spent foraging decreases with more animals coming to the huddling group (Vickery & Millar 1984), or parasites can be transmitted more easily (Gilbert et al. 1010).

3. MATERIALS AND METHODS

The experiments will be carried out at the Research Station of Göd on fifth generation descendants of mound-building mice caught in the wild, but laboratory kept house mice of can also be used. The mice are maintained under reverse day-night light conditions, that enables the observation of the the active period of this nocturnal animal in the daytime. Test boxes and the tools for handling the animals are provided at the station. Data recording is done manually on previously printed data sheets. For timing of the observations a stopwatch is needed. Data will be transcribed to Microsoft Excel spreadsheets, and analyzed by INSTAT statistical program.

4. PROCEDURE

4.1 The goal of the practical:

During the practical we address the following question about huddling behavior in mound-building mice:

- *Does kinship influence the huddling*?

We expect that closely related individuals will huddle more likely than non-kin mice.

We plan two experimental groups:

- kin (sibling) mound-building mice
- non-kin mound-building mice

Variables to be measured: the number of huddling individuals, the number of mice hanging on the wire-mesh of the cage, the number of mice on the bedding, and the number of mice in the bedding.

During the test we will select four individuals from same aged litters (the non kin group from four different litters) and place them in the test box. The duration of the test is 60 min between 10 and 11 o'clock in the morning. We record the position of the mice at the beginning of the test, and after this in every 15 minutes.

1. Determination of sex in mice

2. Observation of mice with different levels of relatedness under identical circumstances, looking for differences.

4.2 Steps to be done during the practical

1. The demonstrator places four mice from each group into separate boxes on the day before the practical: four kin mound-building mice, and four non kin mound-building mice from different litters. All mice are approximately 60 days old at the time of the test.
   - **Planning of the experiment.** All students plan the experiment with the help of the document: „Necessary and sufficient steps of a study" wich is provided by the demonstrator.
   - Formulating of an experimental question, alternative hypotheses and predictions.
   - Define variables to be collected
   - Choose the statistical method to be applied.

   With the help of the demonstrator the students assemble the experimental groups from the boxes separated the day before: four animals will be put to one testing box, meanwhile the students will practice how to handle a wild mouse safely. After this they will learn how to determine sex in mice. The assembly of the groups is followed by 15 min acclimatization time, which will be used to finalize the plan for the data collecting procedure.

2. The test begins with reading the temperature of the experimental room from a thermometer. One student goes to one of the four test boxes, and records the position of the animals at time 0 other three students record the other boxes. Another student records the variables describing the status of animals at 15, 30 and 60 minutes on the prepared datasheet.

Test design

Timing: between 10- 11 o clock in the morning

Temperature: 21 °C

Experimental groups:

- kin mound-building mice (four individuals)
- non kin mound-building mice (four individuals)

The two experimental groups will be tested simultaneously.

Age of animals: 60±5 days

Sex ratio: 1:1

Summary

Preparations:

Animals should be separated one day before the experiment to individual cages.

15 minutes before the experiment the mice should be placed to the experimental boxes, as a result there will be four experimental boxes which we will observe simultaneously. The boxes are to be separated visually from each other with a non-transparent plastic panel. The experimental boxes contain only wooden shavings as bedding.

Experiment:

The duration of the test is 60 min; we record 5 times the position of the animals on the datasheet; at 0 min, 15 min 30 min, 45 min, and 60 min.

Variables:

- the number of huddling individuals
- the number of mice hanging on the wire mesh of the box,
- the number of mice on the bedding,
- the number of mice in the bedding.
- number of huddling groups (mice can be huddled by two)

1. **Data input:** The students enter the data into an Excel spreadsheet according to the given example. They calculate the means and the standard deviations with the help of the software.

2. **Data analysis:** the data will be transferred from Excel to the INSTAT statistical software.

3. **Visualization of the results on charts:** The means and standard deviations should be presented on a column chart drawn by the Excel software.

4. **Statistical analysis of the data:** In order to decide if the behavior of the mice statistically differs between the groups, we have to analyze the data. We use the INSTAT statistical software. Since we compare two independent groups, we use Student t-test. We give the results of the statistics in form of: t(df)=…, p=….. with the help of INSTAT statistical program

5. **Conclusion and discussion:** From the results of the experiment conclusions should be drawn on the huddling behavior of mound-building mice. Some of the alternative hypothesis can be accepted and some of them will be rejected. When writing the discussion of the report, the following questions should be addressed:
   - In what extent were the results congruent with previous results in the literature?
   - If not, what can be the explanation?
   - What kind of new questions arose during the data collection and the analysis of the data?
   - What would be the next step, what kind of new experiment could be planned based on these results?

Figure XIII.1: Data sheet for the huddling experiment

Figure 13.1 Datasheet for the huddling experiment

Experimenter:

Date: Temp: Daytime:

group :Kin MOUND-BUILDING MICE	minutes	On the wire (nr)	On the bedding (nr)	In the bedding (nr)	Huddling (nr)	comments
	0					
	15					
	30					
	45					
	60					

group: Non-kin MOUND-BUILDING MICE	minutes	On the wire (nr)	On the bedding (nr)	In the bedding (nr)	Huddling (nr)	comments
	0					
	15					
	30					
	45					
	60					

Comments:

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Chapter XIV. Factors affecting the shoal formation in the zebrafish (*Brachydaniorerio*)

Péter Pongrácz

1. OBJECTIVES

During this practical the students can examine some aspects of group formation in a social species, the zebrafish. We perform experiments, which are designed to test the basic factors that influence the willingness of joining to other group members. The goal is to detect those visual peculiarities of an artificial fish simulation, which attract the zebrafish (in other words, factors that may contribute to the conspecific recognition). This practical involves non-invasive testing of living animals, where students collect empirical data, analyse them and discuss the results individually.

2. INTRODUCTION

2.1 Costs and benefits of living in a group

It would be hard to find such taxa in the animal kingdom, where some of the basic features of social behaviour, group formation, moving, feeding, resting in groups would not be present. There are obvious differences among the taxonomic groups in the level of sociability – group living is much more common for example among the birds and fish than among amphibians or reptiles. Ontogeny also can have profound effect on the group formation in particular species, as in frogs the larvae (the tadpoles) usually live in larger groups, meanwhile the adult frogs are usually solitary animals. Seasonal changes of sociability are also common – many birds are strictly territorial during the breeding season, then they congregate into larger flocks throughout the cold (non-reproductive) season.

There are several theories that try to explain why **group formation is an adaptive strategy from an evolutionary aspect. The three most important of these are (1) reducing the risk of predation; (2) more effective foraging, and (3) enhancing the success of breeding**[1] (Krause & Ruxton, 2002).

Forming a group is one of the basic antipredator tactics. There are several hypotheses of how the group may lessen the success of a predator. Maybe the simplest such mechanism is the so-called **dilution effect**. To understand it, imagine a lonely animal when it meets with a predator. The chance of an attack on this potential prey is 100%, till it is alone. However, if another joins, the risk of a predatory attack on that particular animal immediately drops to its half. As the group grows, so does the relative safety of the individual members (of course, with some restrictions: the group should consist of similarly looking and equally healthy/ strong specimens for example). A strongly resembling mechanism to the dilution effect is the **confusion effect**. The latter means that predators usually pick their target not so easily when there is a multitude of potential prey animals in a group. For example when a peregrine falcon is attacking a large flock of starlings, its task is not only choosing one from the many, but also the predator should be deadly accurate at the moment of impact. At a speed of an attacking falcon any miscalculation of the exact collision with the prey can result in a fatal injury on the attacker's side as well. Groups of course can perform more active antipredator behaviours than the previously mentioned passive mechanisms. **Enhanced vigilance** for instance works on the principle of 'more eyes see more'. One of the most time consuming activities for almost every animal is the regular monitoring of its environment, looking for potential predators. When an animal is alone, vigilance has a great cost: while searching for predators, one cannot forage, rest, or do other important activities like courtship etc. When the individuals form a group, even if their vigilance activities are not coordinated, the antipredator monitoring will be more effective and less costly at the same time. There are species (like the meercats or the scrub jays) where an even more developed mechanism was evolved, which includes assigned **sentinels against predators**. Sentinels have only one task while they are on duty: looking for danger. During this time the

[1] See also in Chapter 13 (Huddling)

other group members can concentrate to any other activities. Obviously, sentinels are regularly switched by other individuals[2].

Living in groups can enhance the success of foraging, which can be a consequence indirectly of the reduced risk of predation. In other cases acting as a group provides opportunity for the individual group members to access such food sources that would be impossible or very hard to obtain alone. The evolutionary success of several predatory species was secured by the emerging of cooperative hunting. Gray wolves became the most successful predators of the Northern Hemisphere mainly because of being able to form packs (groups of rather small number of usually closely related individuals), which hunt very effectively on much larger hoofed prey animals. A similar case can be found among the Felidae, where the lion represents the most successful species of the large cats – and again, it is a highly social group hunter of large prey species. Among the aquatic predators we can find further exemplars, like the killer whales and humpback whales that developed very successful cooperative hunting tactics against various prey animals, like smaller fish, seals and penguins. Finally humans should not be forgotten either. Probably the most important species-specific feature of the human race is the extraordinary capacity and ease of forming cooperative and cohesive groups. This inherited capacity is thought to be the key for the evolutionary success of our species, helping us in many aspects of survival, like foraging, defense and reproduction as well.

From the aspect of reproduction those cases of group living are probably the most interesting, where the animals form a group just for the mating period. Sometimes this does not involve more complex forms of social behaviour, because the many times enormous masses of individuals are congregating as a consequence of common and limited occurrence of breeding locations. It is a well known phenomenon in Hungary for example, when in early spring terrestrial frogs and toads are migrating to the local ponds and lakes, forming considerably sized congregations along their route and at the water. Functionally similar, however from ecological and economical aspect much larger scale migrations occur in the seas, when either the herrings, or particular salmon species mass-migrate to their spawning areas. Another typical form of social relationships from the reasons of reproduction can be considered, when the animals show specific sexual/ courtship behaviours after establishing the groups. A good example for this is the common courtship ritual (or 'lekking'), which can be observed for example among the wild turkeys. In other cases the most obvious social behaviour in the mating season is the widespread and many times ritualized fighting usually among the males (like in the red deer, or in the brown hare). Let it the courtship or the fight be the observed activity of the congregated animals, forming a group for the time of breeding serves the purposes of mate choice, in other words it is **an evolutionary mechanism of inter- or intra-sexual selection**. Although fight and courtship behavior may seem to be very different for the first glance, they are close to each other from the functional aspect, and the difference is in their background mechanisms (female choice vs. direct competition between the males)[3].

Finally, it is necessary to mention those factors too, which represent the **costs**, or even the **dangers of group living**. Predators can be attracted to a group of individuals more than to a lonely specimen. A large group can face the problems either of the **limited food, drinking water and room for resting**. Competition for the resources can be fierce, and the distribution of the resources may be very uneven, especially when there is a relevant difference of strength / rank among the group members. Spreading infectious diseases is more frequent between group members, than in a population of scattered individuals. Epidemics are only one factor among the many negative aspects of extreme group densities that characterize the populations of some species during **gradation**. (Gradation is a periodically repeating, fast enlargement of populations via extremely effective reproduction, in good environmental conditions.) Perhaps the most dramatic downfall of group living is the inevitable collapse of these super-dense populations after reaching the climax of gradation.

2.2 Shoal formation in fish

Living in groups is exceptionally wide spread among fish species compared to other vertebrates. Approximately one fourth of all the fish species live permanently in groups, and half of the species form at least temporary groups during their lives. For describing groups of fish from a functional/ formal point of view, we use two slightly distinct terms. When many fish move together in a synchronized manner (same direction, same speed), the name of this formation is "school" (Aoki, 1980). If the group members do not show high levels of synchronization at a time, but they are loosely stay together, this type of group is called as a ***„shoal"***. Obviously, there is a mutual interchangeability between schools and shoals, because when the fish move from one place to another, the shoal will alter to

[2]See also Chapter 15 (McDonalds)
[3]See Chapter 9 (Sexual dimorphism)

a school, and when the fish spend a longer time somewhere with foraging or resting, the school will become a shoal. For the fish, group living comes with the same types of costs and benefits as we discussed it previously in the general introduction (Krause et al., 2000).

2.3 Investigating shoal formation in the zebrafish

2.3.1 The zebrafish

The zebrafish *(Brachydanio rerio)* (Figure XIV.1.) originally lives in the East-Indies, however it became long ago a well known, commonly kept species among the aquarists. It is small (body length is about 6 cm), easy to breed (if it is kept in ideal conditions, they can spawn in every 10 days, and they lay 50-100 eggs at a time); peaceful with other fish and it is even pretty to look at. Besides the aquaria of the enthusiasts, the zebrafish became a favorite subject of a multitude of scientific research as well. Just as the other members of the Danio fishes, zebrafish live in groups in their entire life, basically right after they start to swim first time in their lives.

Figure XIV.1: A group of adult zebrafish

2.3.2 Zebrafish in the biological research

It may seem surprising that a fish can become such a widely used subject of the biological laboratories as some of the rodents, or the fruit fly. However, the zebrafish is a popular research subject around the world, and especially the geneticists use it for testing the effect of mutagens, or various environmental factors that affect gene expression. Among the vertebrates the zebrafish was among the first few species, of which the full genome was sequenced. The zebrafish is an ideal subject for investigating the early ontogeny and ontogenic deviations, as the larvae of this species are completely transparent, thus the development of the inner organs are well visible. Another relevant research field where the zebrafish is among the leading subjects is the study of **lateralization** (Halpern et al., 2003). In a broad sense lateralization means such processes of the neural system, which usually are expressed also on the level of behavior, and can be characterized with a well defined left-right asymmetry.

2.3.3 Testing social behaviour of the zebrafish in the laboratory

When talking about social behaviour in the zebrafish, scientists usually narrow their scope of interest to the shoal forming, in other words social attraction and the regulation of maintaining group cohesion in this species. This phenomenon is primarily not important anymore from the point of view of zebrafish-ethology. Instead, the dynamics of zebrafish shoals (social attraction to conspecifics) offers an easy-to-test model for investigating such factors that can affect even human behavior. Not surprisingly, zebrafish are often used in human-related genetic, physiological and neurological research. Zebrafish have relatively simple nervous system, their genetic setup is well known, and additionally there are no strict rules from the aspect of animal welfare when lethal or seriously invasive experiments are conducted on fish. Recently such testing procedures were invented, which unify the benefits of easy manipulation of fish social behavior with the automated data collection and analysis. Here we provide basic details of this test apparatus.

- The subjects (one or more zebrafish) are placed into a small aquarium. The aquarium contains 15 cm deep water, nothing else.
- To the opposite sides of the aquarium two flat screen computer monitors are placed. These serve as channels of stimulus presentation for the fish in the aquarium (see Figure XIV.2).
- Fish are presented with visual presentations from a computer. These presentations show usually 2D images of other fish, which move back and forth horizontally (like as they would 'swim').
- The most interesting aspect of the fish' behavior in this type of device is whether they form a tighter shoal as a reaction to particular presentations on the monitors. For describing the group's behavior, a few key variables are collected. If the presentation is showing such fish images, which attract the subjects, they swim closer to that monitor (supposedly for joining to the projected 'fish', forming a larger shoal). Consequently, the distribution of the zebrafish in the test aquarium will be unequal, as the subjects are drawn to one of the monitors. Another important parameter to describe shoal formation is the inter-fish distance within the group. Obviously, when the subjects form a tighter group, the distance between them will be shorter. Independently of their actual location in the aquarium, the zebrafish swim closer to each other if they are presented with a fear-eliciting object (like a predator image) from above, or on some of the monitors.

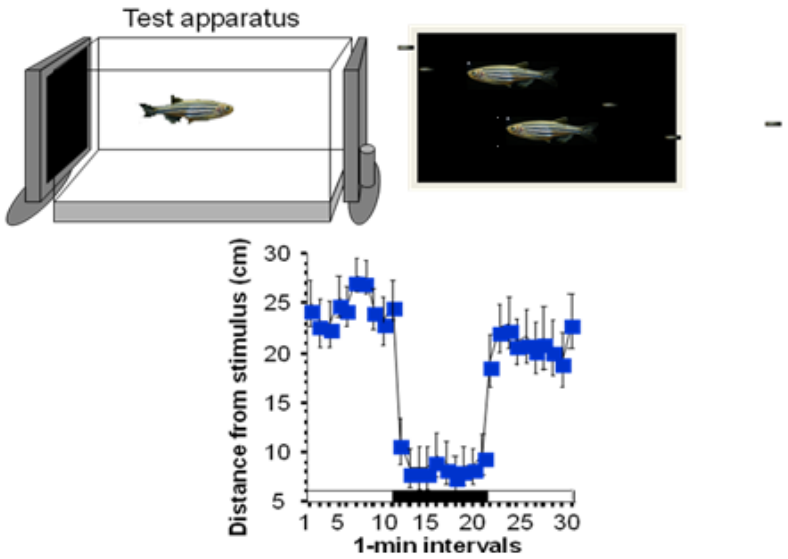

Figure XIV.2: Schematic picture of the testing apparatus for following group forming behaviour of the zebrafish (based on Gerlai et al.). One or more zebrafish are placed into a small aquarium, which is equipped with two flat screen computer monitors along its opposite walls. Through the monitors researchers show different 'fish presentations' to the subjects. On the graph at the bottom, the results of such an experiment are shown, when images of zebrafish were shown on one of the monitors (black stripe along the x-axis). The subject swam closer to the presentation, and it is detected through the shortened distance between the fish and the wall in front of the monitor.

2.3.4 Factors affecting the group formation of zebrafish

Researchers of several laboratories worked on the details of the mechanism and ontogeny of group behaviour in the zebrafish. They described several neurophysiologic factors that underlay the social behavior of these fish. It turned out that ontogeny of group behavior takes a different course between particular inbred lines of zebrafish. In a series of applied studies such methods were developed, which enabled the researchers to employ zebrafish' group behaviour as indicator of the adverse effects of alcohol on humans.

The typical between-fish distance in adult zebrafish is about five-six times of their body length. This distance is not affected significantly by the size of the aquarium, in other words a group of adult zebrafish will be approximately the same size in a very large or in a smaller tank. Juvenile fish behave differently. Young zebrafish 'use the space' if they can, in a large aquarium they scatter to a bigger extent than the adults. The between-fish distance settles to the usual five-six times body length when the fish reach half year of age (Engeszer et al., 2007).

Among the environmental factors that probably affect group cohesion the effect of food and predators were tested. If food was scattered in the test aquarium, zebrafish loosened the group (the between-fish distance increased).

When a hawk-like silhouette was 'flown' over the aquarium, the fish reacted initially with a quick dispersal. After about a half minute though, the group pulled together, with a shorter between-fish distance than prior to the predator presentation (Suboski et al., 1990).

What are those factors (key stimuli), which initiate **social attraction**[4] in the zebrafish? A logical hypothesis would be that the conspicuous horizontal stripes of this species play a role in recognition of conspecifics. In an experiment where the subjects were shown presentations of zebrafish-sized fish images, wearing horizontal or vertical stripes, it was found that the arrangement of the stripes does not affect social attraction in the zebrafish (see Figure 3). When researchers manipulated the colour of the fish stimuli, an interesting phenomenon was found: zebrafish were attracted stronger to the conspecific images that showed yellowish coloration instead of the natural colour pattern. This result can be regarded as an effect of a **supernormal stimulus**. In these tests always adult female fish are used, that are especially attracted to the golden bands appearing on the male zebrafish. Therefore the yellow fish images may attract the female subjects through sex-specific channels. These results can be summarized that pattern and colour are not very important for zebrafish as elicitors of social attraction. However, when other experiments used artificially altered shapes of zebrafish images (elongated or shortened), this caused diminishing social attraction in the subjects (Saverino & Gerlai, 2008). This gave us a proof that the contour (and perhaps the size) of the other fish is important to elicit social attraction in the zebrafish.

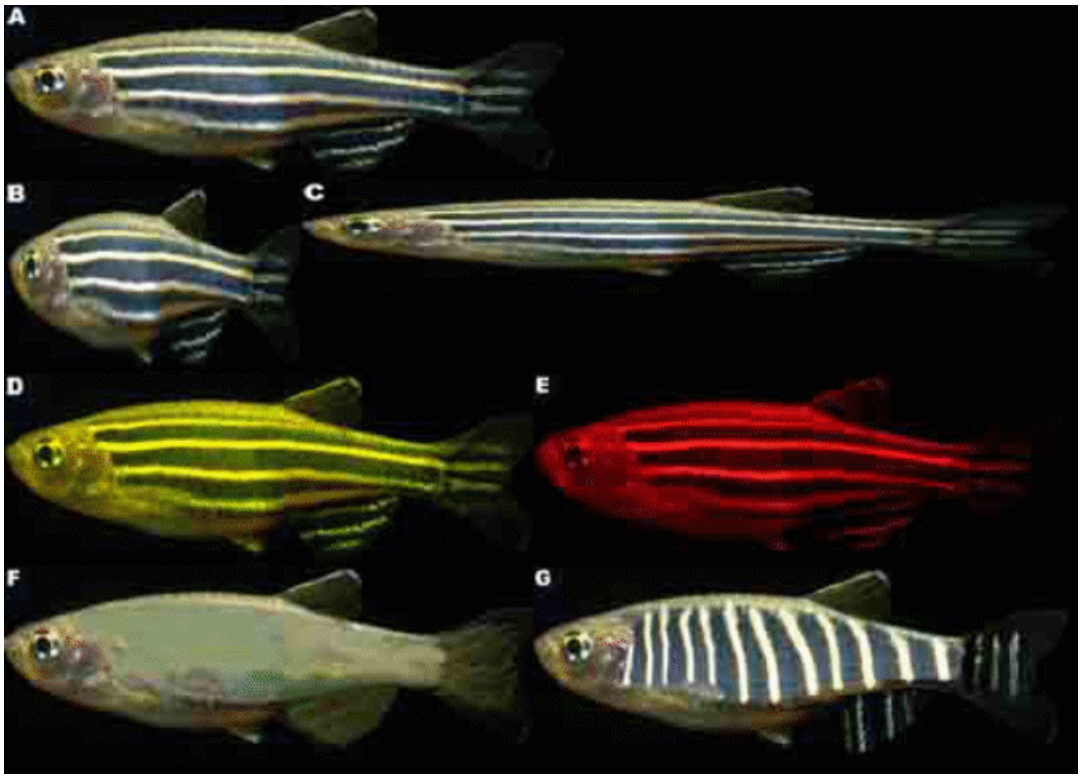

Figure XIV.3: Different types of fish-stimuli that were used as presentations for zebrafish to elicit social attraction (based on Gerlai et al.). A: natural looking zebrafish; B: shortened image of a zebrafish; C: elongated image of a zebrafish; D and E: yellow and red colour variants; F: fish without stripes; G: fish with vertical stripes.

The connection between particular **neuro-transmitters** and the ontogeny of shoal formation was discovered with neurophysiologic experiments. For this purposes the brains of fish from different age classes had to be removed and the concentration of the neuro-transmitters had to be measured. The concentration of the **dopamine and dopamine-like transmitters** showed similarly growing curves as the willingness to form a shoal in zebrafish with the age (Buske & Gerlai, 2012). When fish were treated with a chemical that blocks the dopamine D1 receptors in the brain, fish stopped to form a shoal (while their vision and moving ability, along other important behaviors, remained unaffected (Scerbina et al., 2012)). There are also other results that support the connection between the **dopaminergic system and social behaviour**. In an inbred strain of zebrafish (named as 'AB' strain) the willingness to form a group grows steadily along the ontogeny. At the same time in the 'TU' strain there is a sudden increase

[4]See also Chapter 10 (Huddling)

of group forming between the ages day 25 and 50. By measuring the dopamine concentration in the brains of the fish, the results showed a linear increase in the 'AB' strain, while in the 'TU' strain the dopamine level has a steep increase along the ontogeny (Scerbina et al., 2012).

Our last example is about how zebrafish became the model for testing the effect of alcohol on social behavior. Embryos (while still in the egg) were treated with different physiological densities of alcohol-water solutions (0.25%-1.00%). It turned out that the membrane of fish eggs provides considerable protection against the alcohol diffusion. When the treated fish hatched, they were raised and tested as adults in the above described testing apparatus. When they were presented with images of conspecifics, it was found that even the lowest density of alcohol solution weakened the social attraction towards the zebrafish presentations. Fish that were treated with the highest density of alcohol (1.00%) were absolutely not attracted to the images of conspecifics. With proper control experiments it was also shown that the effect of alcohol on the deterioration of social attraction was not caused by side-effects on the fish' visual sense or their motoric functions (Gerlai et al., 2006; Gerlai et al., 2008).

3. MATERIALS

3.1 Subjects

The experiments will be conducted on adult female zebrafish. In Experiment 1 a single fish is placed to the testing aquarium, and in Experiment 2 four fish will serve as subjects.

3.2 Testing aquarium

The testing aquarium is a 20 l tank, filled with water 15 cm deep. No any plants or other objects are present in the aquarium. The water temperature is between 22 and 24 Celsius degrees. At the two opposite ends of the aquarium two flat screen computer monitors are placed, positioned tightly as possible to the aquarium walls. The monitors serve as the source of visual presentations for the subjects within the aquarium – the presentations are sent to the screens from a computer. The aquarium is divided to sections with lines painted on the bottom of the tank, 5 and 10 cm away from both sides where the monitors are. A fifth line marks the middle of the aquarium (see Figure XIV.2).

3.3 Experimental groups

Students perform two experiments. In Experiment 1, we will investigate whether a lonely zebrafish would prefer its conspecifics (presented on a monitor screen) over a group of similar sized heterospecific fish. We send a simultaneous presentation of five adult zebrafish and five adult platys (*Xiphophorus maculatus*) to the monitors as test stimuli.

In Experiment 2, we use artificially manipulated images of adult zebrafish as test stimuli, and our question is whether a group of zebrafish reacts differently to particular key stimuli of conspecifics. Four fish will be placed to the testing aquarium, and one of the monitors will show the test stimuli. These will be (separately presented, in a randomized order) (1) natural type zebrafish; (2) natural markings but yellow instead of silver stripes; (3) natural coloration, but vertical instead of horizontal stripes; (4) natural coloration and pattern, but three times longer elongated fish silhouette. In each case images of five fish from the same type will be presented on the monitor.

4. PROCEDURE

4.1 Experiment 1: testing social attraction to conspecifics

A single fish is placed to the test aquarium with a small net. It is important to let out the fish gently to the water by submerging the net and not dropping the subject to the water from the air. Before starting the presentation on the monitors, we should wait for 10 min, giving enough time to the fish for familiarization with the environment.

Students work in pairs. A recommended sharing of tasks for example: student 1 operates the computer/ presentations and watches the behavior of the subject, while student 2 writes the behavioural parameters to the data sheet. A

presentation lasts for two minutes. Each subject receives eight presentations as a total, with 2 min breaks between the presentations (during the break the monitors are bleak). Presentations of the conspecifics (zebrafish) and the heterospecifics (platy) will occur from left and right in a changing order. Students may switch their roles at the half of the experiment, giving opportunity to each other to perform each part of the test. The behavior of the fish is recorded during each presentation. The following parameters are to be collected:

- Latency (s) of entering the 10 cm sections (120 s, if the fish does not enter at all)
- Latency (s) of entering the 5 cm sections (120 s, if the fish does not enter at all)
- Number of entries to the 10 and 5 cm sections
- Total time spent in the 10 and 5 cm sections
- Total time spent on the left and right half of the aquarium.

At the end of the experiment the subject is transferred from the testing tank to a common keeping aquarium. This ensures that each fish is tested only once.

4.2 Experiment 2: testing phenotypic features that may affect social attraction in the zebrafish

Four subjects are placed to the testing aquarium. We leave them there undisturbed for 15 minutes, to let them habituating to their new environment.

We show four separate presentations to the small group of subjects. Each presentation consists of the image of five similar fish, and it is shown on one of the monitors only. Presentations are shown from the left and the right in an alternating order. Each presentation is 2 min long, and between them we leave 2 min long breaks (the monitors are bleak). As a control, we start the recording of the subjects' behaviour 2 min before the first presentation. Data collection is done in 10 s long intervals. The following parameters are to be collected:

- Number of fish in the 5 and 10 cm sections
- Number of fish on the left and right half of the aquarium

4. 3 Data analysis

4.3.1 Experiment 1

During the analysis, we compare the zebrafish' behavior between the vicinity of the two monitors, on the basis of the type of the presentation (zebrafish vs. platy). For this we have to create data columns from the eight presentations, separately for the parameters (latency, total time spent, number of entries), and the presentation type. As the data originates from the same subject, the zebrafish vs. platy comparisons have to be analyzed with repeated tests. Whether the data shows normal or non-Gaussian distribution, we use paired t-test or Wilcoxon signed rank test, respectively. Finally, we create visual illustrations of the results, where we can show the platy and zebrafish data in a well comparable manner.

4.3.2 Experiment 2

During the analysis we compare the parameters between the different types of fish presentations and the control period. As we wrote down the number of fish in every 10 s, from each 2 min period we will have 12 data of each parameter. We arrange these to data columns, and compare them separately for each parameter. Remember that the presentations were shown on only one of the monitors at a time, so we should arrange the data as 'presentation's side' and 'bleak side' instead of 'right' and 'left' side.

We analyze the data with repeated procedures, as the same subjects were tested again and again. Depending on the result of the normality tests, we use ANOVA for repeated measures in the case of Gaussian distribution, or Friedman test (not-Gaussian distribution); with appropriate post hoc tests subsequently. In the case of significant main effect the post hoc test shows which are the groups that differ significantly from each other. Finally, we have to create visual illustration for the results. Such graph style should be chosen, which gives a good opportunity for the reader to compare the behaviour of the subjects in the case of the different stimulus presentations.

4.4 Evaluation of the practical report

- Did the student write a detailed introduction, including the scientific background of the research, the experimental question and hypotheses?
- Did the student explain the methods and materials of the experiment?
- Were the necessary statistical analyses performed and presented in the report?
- Were the results illustrated with acceptable graphs/ figures?
- Did the student explained and discussed the details of the results?
- Were the mathematical formulas and statistical analyses correct?
- Does the report include a general discussion, where the student draws the broader conclusions of the study, and connects the new results to the former knowledge based on the literature?
- Does the report fit to the formal and aesthetical requirements?

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Chapter XV. Aggression and dominance in the house mouse

Vilmos Altbäcker
Péter Szenczi

1.OBJECTIVES

During the practical students get insight on the processes of group forming, aggression, rank and dominance order. The main objectives are to practice measurements of behavioural characteristics related to experiments on quantifying level of aggression within groups. In these kinds of experiments it is very important to execute them fast and precisely in order to cause minimal stress to the animals. An animal's reaction to certain situations is closely related to its internal hormonal state. Every artificial or extra impact can modify the results of such test especially in social encounters. The second objective is that students gain experience in analyzing video recordings, recognizing behavioural elements and traits.

2. INTRODUCTION

2.1 Group formation

Most mammals live in groups at least for a short period during their lifetime; therefore they show some kind of social behaviour. In general, groups are being formed because all participants realize fitness benefits, for example better survival or increased reproductive success. The simplest types of groups, which are not formed by some kind of attraction between individuals but other factors, are called a**ggregations**. Such factors are like common migration or the attraction to a temporarily existing resource location. If there are no real relations between individuals it is called an **anonym group**, if connections exist it is called an **individualized group**. Animals can join and leave **open groups**, while members of a **closed group** can recognize each other and are intolerant to strangers.

Animals aggregate mainly because of the availability of certain resources or to reduce predation risk . Groups can be temporary or stable, with or without inner structure.

Group living can improve the feeding success of an individual due to more effective defence of territories, better access to information on good feeding sites or the possibility to hunt larger prey by cooperative hunting . Moreover, the dilution effect (decreased probability of being taken by a predator) and increased attention (Roberts 1988) improve protection against predators.

Social partners are important environmental elements since they are potential mates and participants in cooperative and competitive interactions. The formation of groups and the related behavioural patterns have both costs and benefits. Living in groups allows the development of complex social behavioural traits like alarm calls, food sharing, helping, communal breeding, establishing dominance order, individual recognition and mating systems, which further increases the gained benefit. However, there are numerous disadvantages as well; competition is higher, which leads to elevated aggression ; while dominant individuals may also monopolize the resources[1].

2.2 Aggression

Aggression is when the individuals try to limit each other's access to a certain resource. This phrase is used on a wide variety of behaviours. *Sensu stricto* it is used when the aim of the behaviour is to inflict physical injury. *Sensu lato* aggression is when an individual suffers disadvantage because of the actions of another specimen.

In most cases within group aggression manifests itself in a ritualized way, during which opponents get information on each other's strength and establish a rank order to obtain their share from resources. Such a hierarchy prevents

[1]See Chapter 13 on huddling

the further fights in later encounters when the subordinate individual waits until the dominant uses the resource. In most cases signalling the social status is enough to solve conflicts. However when odds are near to equal, ritualized aggression can turn into real fighting. Since in real life conditions the weaker competitor is able to flee, the fight seldom ends with serious injuries.

Level and manifestation of aggressive behaviour is closely related to a species' social system and distribution of resources. Random distribution of resources is usually exploited by territorial behaviour, while patchy distribution leads to groups with dominance order. There is a close connection between a population's social system, forming and maintaining of groups and the ecological constrains, the distribution of resources, which define the level and target of agonistic behaviour. Mutual tolerance between individuals is essential for behaving cooperatively, while selective aggression directed toward strangers may be very important in maintaining territories and protecting resources.

2.3 Social rank

Group living enhances competition between individuals which leads to elevated aggression. In order to avoid spending too much time with fighting, in individualized groups hierarchy order is established. The rank is the result of dyadic interactions. In most cases the outcome of a fight is based on the difference of physical size of the opponents, but some cases experience, possessing certain traits, or reproductive status can have a great effect on it, too. In very rare cases the position in the hierarchy can be inherited as well. It is important to note, that previous experience greatly influence an animal's behaviour in agonistic interactions. Those that have already won such battles are more likely to remain victors, while those that lost their first fight remain losers. The ranking, also known as hierarchy, can be interpreted as a kind of prediction of the most likely outcome of aggressive encounters between particular individuals.

In the case of a so-called linear rank order, from two individuals there is a dominant and a subordinate. The dominant gets more or better quality food, or has the opportunity to copulate more and therefore it will have more successors. However, to achieve a dominant position the individual takes more risk, spend more time fighting and has a greater chance of injury.

Hierarchy among individuals is like a ranking in a championship. In a linear hierarchy position of each animal is definite, there are no draw, or network of rankings where two or more individuals are on the same level. To name positions we use Greek letters, first is the alpha, the second is the beta etc. On the bottom of the hierarchy there is the omega individual.

Linear hierarchy is also called as the pecking order. Originally it was described in groups of domestic chicken hens, where fighting manifests in pecking on each other. This kind of social structure is best observable in groups with no more than 10 individuals. In a competition for a certain resource the lower ranking individuals always retreat when facing a higher ranking one. If not, the dominant start showing aggressive behaviour. It is expressed only by ritualized signals at first, then - if that was not sufficient enough - in real fight.

Hierarchy is dynamic, as position is related to physical state. As an animal grow older and weaker, its position eventually drops also. Such system is typical for the social systems of group living monkeys and apes.

2.4 Communication and rank

Many species of mammals live under complex social conditions, where communication between individuals is unavoidable. Signalling status is an important part of communication. It can be done via sounds or visible signals, but in mammals for example perhaps the most common way is to use olfactory cues.

The evolutionary background for this is that the first mammals may have been nocturnal creatures, and smelling played a major role in their communication. Using chemical signals has many advantages over visual or auditory communication. It can be used when other signals are hard to detect, like in the dark or in dense vegetation. Odours can give information about an animal's movement in space and time. The signs last longer and do not require the presence of the signaller either.

Odours used by the mammals are not equivalent to the pheromones used by the invertebrates. The mammalian chemical compounds usually have a much more complex chemical structure, and the triggered behavioural response

depends strongly on the context and the receiver's prior experience. Hence the proper phrase is 'social odours' for the chemical signals of the mammals[2].

2.5 The Social system of the house mouse

The house mouse (*Mus musculus domesticus*) is one of the most widely used species in behavioural, physiological and genetic experiments. It is an ideal laboratory species because keeping them in captivity is easy and they are breeding fast (mature in 2 months). A further advantage of the house mouse is that its genetics is also well known.

If someone wants to study mice, there are many well documented and reliable experimental protocols to start with. The behaviour of its wild populations as well as many inbred strains under laboratory or semi natural conditions is also studied profoundly .

The house mouse is a commensal species; it lives with humans in close connection across the majority of its range. It can be found in very high densities when conditions are favourable for it reproduction. Under natural conditions males keep territories shared with several non-territorial females. Males defend actively the borders of their territories; hierarchy – which in turn related to breeding possibilities – is only established among females. Usually the older females are dominant over the younger ones. In very high densities maintaining distant territories is not possible anymore.

Under good conditions when there is plenty of available food – like in many cases in the human settlements – individuals tolerate each other, but that does not mean that they all have the same share of the resources. Some highly aggressive mice can defend a territory, but the others must share the remaining space with their conspecifics. After the hierarchy is established, the individuals' access to resources such as food and mates are determined by their rank.

3. MATERIALS AND METHODS

3.1 ANIMALS

Animals are descendants of wild caught mice kept at the Biological Station of ELTE at Göd. They are housed under standard conditions in regular sized mouse boxes. Temperature is kept constant (between 18^{o}C and 21^{o}C) and reverse 12 L: 12 D light/dark cycle with red light between 0800 and 2000 hours was set up. The reversed light cycle is necessary for this nocturnal animal being active during 'normal daytime' when the experiments are performed with them.

3.2 METHODS

The tests are carried out in a 50 x 30 x 35 cm glass terrarium. The cage is divided into two equal parts by a plastic partition wall. Before the practical, all animals are kept solitarily; therefore their social status is neutral. At the beginning of the test, we weigh the subjects and place them to the opposite sides of the cage, and left undisturbed for five minutes. Then we remove the central partition, and start the video recording. The test starts when one or both animals approach the other for the first time. Beginning from this time, the whole test lasts for 10 minutes. In the case of fight between the two animals, the test must be stopped if one of the animals is injured or unable to avoid the attacks of its opponent.

We measure the time that the animals spent with agonistic and sociable behavioral elements. Observed behavior units are thus grouped into sociable behaviors (attend, approach, nose, follow, sniff, investigate, grooming), aggressive behaviors (offensive upright posture, threat, boxing, fighting, thrust, chasing) and defensive behaviors (defensive upright posture, retreat, evade, flee, and crouching posture). Latencies of first approach, first agonistic interactions and the identity of the animal first to attack must also be recorded.

At last we evaluate whether the smaller or the larger individual spent more time with aggressive behaviour, and this animal will be considered as dominant as a result of the encounter between the two mice.

[2]See Chapter 7 for related information on chin marking

4. PROCEDURE

The aim of the practical is to evaluate whether the size of the opposing house mice determine the outcome of fights. The experiment is a simplified repetition of the protocol followed by Szenczi et al. (2012), thus we can compare the results with the outcome of the named article.

The practical takes place at the research facilities of the Biological Station. If there are not enough test subjects available, we will use pre-recorded test footages for evaluation. During the test we observe and analyze the agonistic behaviour of mice which are of the same age but they are differently sized individuals. Level of aggression can be characterized by the frequency of certain behavioural traits. By analyzing these, we try to determine the rank difference between the individuals. Scoring the test must be done on a datasheet, data analysis is performed with Excel and Instat softwares.

We start the test by filling out the form „STEPS OF A SCIENTIFIC STUDY" (see Fig 15.1).

The initial question should be answered by yes or no- In this case for example: whether weight of the house mouse determines the time spent with agonistic behaviour and the resulting hierarchy among the fighting individuals?

We formulate also alternative hypotheses. For example: yes, the weight of an animal determines the time they spend with agonistic interactions; or no, the weight of an animal does not determine the time they spend with agonistic interactions

We define the behavioural variables. We decide the start and length of the test.

Behavioural traits to be measured

Time (s) spent with…

- offensive upright posture,
- threat,
- boxing,
- fighting,
- thrust,
- chasing
- evade
- flee
- crouch
- latency of the first agonistic interaction
- individual first to attack

We practice the scoring via watching a few minutes of earlier video footages.

- Measure the weight of the individuals, and place them into the arena. The first 5 minutes is called habituation time, during that the animals are separated from each other. After this we remove the central partition and allow the mice to interact..
- score the test on the provided data sheet
- type the data to MS Excel
- Analyze the data, calculate mean and standard deviation
- Prepare a bar graph of the results (with means and SD)
- Choose a proper a statistical method to analyze the data in INSTAT

The statistical test is used to determine whether the two sets of data are belonging to the same or different population. We use t-test, since we compare two independent groups (heavy and light mice).

Provide the results of the test as: t (df)=…, P =…..

Draw conclusions based on the following questions

- How concordant are the results with previous findings?

- How can you explain the results?
- Ask further questions based on the results

Figure XV.1 *Main steps of the mice experiment*

NECESSARY AND SUFFICIENT STEPS OF MICE STUDY

1/ INITIAL (DECISIVE!) QUESTION:

2/ ALTERNATIVE HYPOTHESES (answers)

A:

B:

3/ EXP DESIGN: **GROUPS:**

LIST POSSIBLE VARIABLES:

DEFINE VARIABLES TO BE MEASURED:

Var1: name-equipment- how-in what quantity

Var2:

Var3:

Var4:

4/ SAMPLING DESIGN

-UNITS / GROUP: n=2V/D2

-SIZE of sample area/LENGHT of test

-distribution: random/even

5/ METHOD/EQUIPMENT TEST: ACCURACY versus RELIABILITY

6/ CONSTRUCT A DATA SHEET: see sample: header, signature, remark, data in one block

7/ OBTAIN DATA: it can save your life!

8/ ANALYSE DATA: A/ t; U; X2 TEST, B/ CORRELATION INSTAT

9/ RESULT (answer) IN ONE SENTENCE:

10/ DISCUSSION:

11/ NEW, IMPROVED QUESTION:

Figure XV.2. data sheet to be used

DATA SHEET for studying....MICE AGGRESSSION……..……..

DATE: **EXPERIMENTER:**

GROUP	VAR	UNIT	Var1	Var2	Var3	Var4	Remark
1		1					
1		2					
1		3					
1		4					
1		5					
1		6					
1		7					
1		8					
1		9					
1		10					
2		1					
2		2					
2		3					
2		4					
2		5					
2		6					
2		7					
2		8					
2		9					
2		10					

Mean group1:

Mean group2:

Standard deviation1:

Standard deviation2:

STATISTICS: **t(df)=…… p=…..**

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Chapter XVI. Group effect on human vigilance during feeding

Vilmos Altbäcker

1. OBJECTIVES

This practice will introduce students to studying human behaviour in public areas. It demonstrates one advantage of being gregarious: the shared vigilance during feeding. Even though our everyday urban life lacks real dangers during dining, or behaviour still reflects the ancient conditions when being vigilant was necessary in an environment full of enemies. Similarly to many animal species feeding in open areas, humans still show regular scanning during the feeding bouts, and although such looking around has no obvious reasons nowadays in the modern societies, it still occurs regularly. We will study if the size of the group around the table, and the openness of the area affect this scanning behaviour.

2. INTRODUCTION

2.1 Group formation as a means to reduce predation risk

Foraging is a risky business especially in open habitats. Most species face some level of predation risk while foraging and any behaviours reducing the risks of being caught while eating should be favoured by selection. Many animals look up and scan the environment while they are eating. This scanning and alert behavior is called vigilance. Vigilant behavior, defined as the frequency and/or duration of scans, can serve many functions (Caraco et al. 1980; Gluck 1987; Lendrem 1983) the best studied one being predator detection (e.g., Lima 1990). A widely studied phenomenon is the “group-size effect”, meaning that vigilance should decrease as the group size increases. Such change has been observed in numerous animals from fish to mammals (e.g., Bertram 1980; Caraco 1979; Godin et al. 1988; Holmes 1984; Roberts 1996; Studd et al. 1983; Sullivan 1984; reviewed by Treves 2000). Even though predation is absent in current urban situations, the effect of group-size has also been observed in humans (Barash 1972, Wawra 1988, Wirtz & Wawra 1986) suggesting that vigilance in humans reflects ancient evolutionary pressures.

Vigilance can help the animal to avoid an unexpected predatory attack by several means. One possibility is that the approaching predator is detected earlier, as several eyes see more, which is called the ‘Many eyes’ hypothesis. This argues that predator screening is shared among group members, thus, the larger the group, a given individual needs to look around the less. Vigilance is a time consuming action, which is in trade-off with several other behaviour including feeding, thus grouping and sharing this task is of adaptive value if other group members are not cheaters (accepting the help of others while not contributing to the monitoring) (Bednekoff & Lima, 1998). Even in cases when predator detection probability is not increased by grouping, the chance is reduced that the focal individual is captured by the predator, this is called the ‘Dilution effect’ (See also Chapter 2).

2.2 Grouping and vigilance in animals

Arenz and Leger (1999a) studied vigilance of ground squirrels (*Spermophilus tridecemlineatus*) and found that the more risky is the antipredator behaviour, the less frequently can it be seen. Later they also added that young animals are less vigilant than adults in this species (Arenz & Leger, 1999b).

Bertram (1988) found that individual vigilance decreases when group size increases in ostriches. There was a sex difference in their behaviour, cocks were more alert than hens. He concluded that individuals benefited from joining to a group as lonely ostriches suffered more attack than feeding groups. Tasmanian devils also show reduced level vigilance when the studied animals were adults, and/or they were in larger groups (Jones, 1998). Marmots seem to be an exception as their group size explained only a fraction of variance in vigilance during feeding (Blumstein, 1996.).

2.3 Group size and level of vigilance in apes

As the above reviewed studies illustrate, most animals show reduced level of vigilance when they are in groups (Roberts, 1996.). Feeding apes move in upright position, which enables earlier detection predators, thus group size may not affect their individual vigilance. As an alternative hypothesis suggests, looking around while feeding may serve conspecific monitoring and not an antipredator function in apes (Treves, 2000). Human groups are especially interesting subjects in this sense as their gaze direction can easily be detected due to the white eye corners (Butterworth & Itakura, 2000.). Looking around in humans is a conspicuous feature which is rather easy to detect, therefore several functional explanations have been developed to explain human vigilance, including predation risk assessment and looking around to follow specific group members like friends or potential partners (Dunbar et al., 2002).

3. MATERIALS

3.1 Studied subjects and necessary tools

We will describe the vigilant behaviour of human subjects during their feeding. As we want to compare behaviour of groups of people in several repetitions of similar situations, a restaurant with many tables should be visited. Observing people during their feeding can be disturbing for the subjects, therefore maximal discretion is necessary. Permission to perform such an action should be asked prior to the practice. We will visit the Western City Alley which contains several restaurants where many people feeds simultaneously and their behaviour can easily be followed from the balcony without disturbance. Select your observation site carefully so that you can easily watch your subjects while they are not aware of being watched. For this reason, we suggest that you sit at least 5 meters away. To test for the group-size effect, observe the scanning behavior of focal subjects (1) eating alone, (2) eating with another individual, and (3) eating in a group of four people. You will need the data sheet (Figure XVI.2-3, see later), as well as a wristwatch as a timer.

4. PROCEDURE

We will test the predictions of several hypotheses explaining vigilance.

a/ Examining the group size effect on the level of vigilance

Based on previous results, we expect that vigilance will decrease by group size. This predicts that as group size increases, the scanning frequency and duration of vigilance are expected to decrease. We will test this prediction by comparing the scanning behaviour of people feeding in groups of different sizes.

b/ Testing the predictions of the Dilution effect and the Many-Eyes Hypotheses

The Dilution effect hypothesis predicts that the chance of being caught by a predator decreases as the group size increases. Thus an individual's predation risk depends simply on the presence of its foraging partners, and its behaviour should reflect this. The Many-eyes hypothesis predicts that the predation risk is actually reduced by the vigilance of foraging partners, and the total amount vigilance is constant but shared among the group members. To separate these alternative hypotheses we can observe how members contribute to vigilance at the group level.

c/ Testing the predictions of the habitat structure hypothesis

This hypothesis predicts that vigilance should depend on area openness. Thus the general level of vigilance can be higher in open areas (large restaurants) compared to small or compartmentalized rooms. The same applies to the local vigilance level within a large area; we expect increasing vigilance towards the center of large rooms compared to places near walls.

We may also consider other alternative explanations suggested while describing other species. Even though the group-size effect has generally been related to predation risk, competition with foraging partners may also result in similar changes by group size. The Conspecific Detection hypothesis predicts that lonely individuals change their vigilance in order to detect other individuals moving around. These explanations refer to other situations and will not be tested during this practice.

4.1 Steps to be followed

We occupy distant observation points to record the behaviour without disturbing people.

The behaviour of guests coming after our arrival will be recorded.

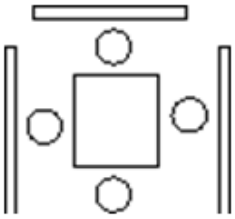

Figure XVI.1 Schematic representation for labeling the position of subjects around the dining table. Circles: chairs, Square: desk, Bars: visual barriers

You should label the actual position and gender of people using the scheme on Figure XVI.1 for each group on the Data sheet (see Figure XVI.2 below)

We will compare the vigilant behaviour of people feeding in groups of 1, 2 and 4. Observation will last for 5 minutes in each group of people. If feeding is discontinued we will discard the observation and start with a new group. We continue the observation until 5-5 repetitions per group size category are completed.

We record the number of scannings, look around behaviours without obvious reasons, within that 5 min period for each person in each group.

Observation is done in pairs and data should be pooled and averaged before the calculations.

Spend the first period collecting pilot data so that you will be familiar of how to record the data and can assess what the problems might be. Collect pilot data for at least one from groups of three different sizes. Then continue with collecting 5-5 sets of data for each group size.

After the observation session, we will go back to the laboratory and analyse the data and finish the report, which should contain the original data sheet.

4.2 Statistical analyses

a/ effect of distance from walls on the vigilance level

b/ effect of group size on the vigilance

Any statistical package (InStat, SPSS, Statistica) can be used to analyse the data. The last two chapters of this volume also help in deciding which methods are to be used. However, on the one hand, people feeding in same sized groups in either open or boxed areas should be compared, and on the other hand, people feeding in similar (open) places but in differently sized groups should be compared to test the group size effect.

4.3 Questions for discussion

- How can we interpret our results?
- Do you have any suggestions for a proper simultaneous analysis of two factors which were separately analysed here?
- What were your impressions: did the genders behave differently? Was there an age effect?
- Do you have suggestions how does the size of the dining room affect vigilance?

Figure XVI.2. Data sheet for studying area openness on vigilance

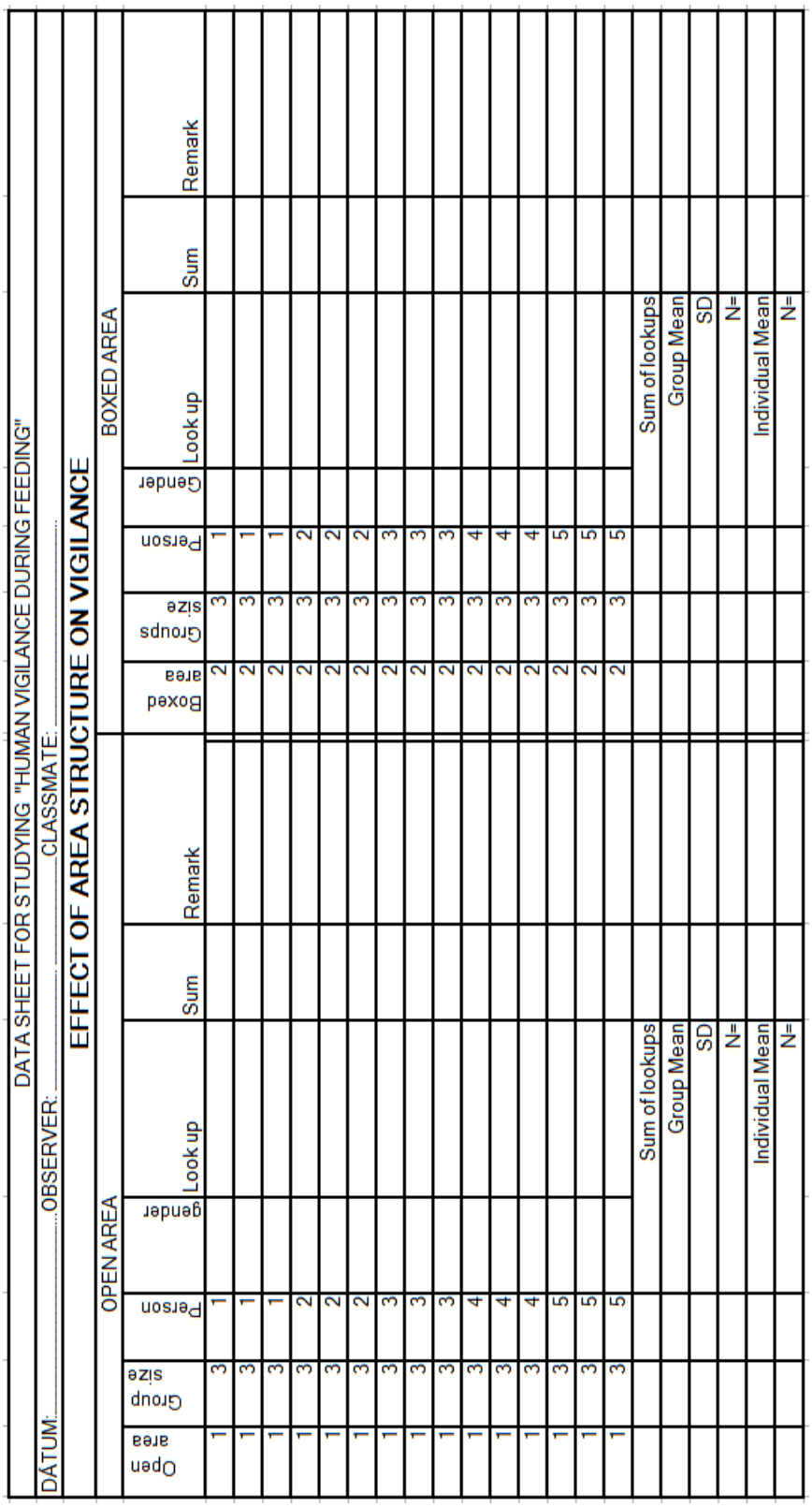

Figure XVI.3 data sheet for studying the group size effect

DATA SHEET FOR STUDYING THE "GROUP SIZE EFFECT"

DATUM:.....OBSERVER.....CLASSMATE.....

FEEDING IN GROUPS OF THREE

Area type	Group type	Member	Gender	Look up	Sum	REMARK
1	1	1				
1	1	2				
1	1	3				
1	1	4				
1	1	5				
1	1	6				
1	1	7				
1	2	1				
1	2	1				
1	2	2				
1	2	2				
1	2	3				
1	2	3				
1	2	4				
1	2	4				
1	2	5				
1	2	5				
1	2	6				
1	2	6				
1	2	7				
1	2	7				
				Sum of lookups		
				Group Mean		
				SD		
				N=		
				Individual Mean		
				N=		

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Chapter XVII. Ethological study of the dog's attachment behaviour

Márta Gácsi

1. OBJECTIVES

Recently dogs became popular subjects of ethological experiments as a natural behavioural model of particular socio-cognitive abilities of humans. This practical is designed to provide students insights into one of the major parallels that seems to serve as a basis on which many crucial human-analogue capacities can be developed; the ability to form individual attachment relationship bonds. Students will be acquainted with the ethological approach of assessing attachment, observing and measuring the behavioural variables that make the objective investigation of such a phenomenon possible.

During the practical live dogs are present and serve as subjects, thus students have the opportunity to try the essence of a method applied by both psychologists and ethologists in their experiments.

2. INTRODUCTION

2.1 Theoretical Overview

Dogs are, inevitably, one of the most successful mammalian species worldwide. Some live in very loose contact with humans whilst others spend their entire life as pets. However, both humans and dogs share an interspecific social environment. In other words, it is natural for them to live their lives with members of the other species: people with dogs and dogs with people. The most striking feature of the social life of dogs is that they seem to prefer joining human groups and this makes this animal special not only as a pet but also as a scientific subject.

When trying to define our relationships with our dogs the phrases that probably come first in many people's minds might include 'the dog is my friend', 'my partner', etc., and vice versa; 'I am his leader', 'he loves me'. Owners often support their beliefs with anecdotal stories from around the world of dogs bonding with people. In the scientific literature, however, this anthropomorphic approach is heavily criticized by sceptics, who consider this view as non-scientific over-interpretations of dog behaviour. Experts often argue that dogs are just domesticated carnivores, originally selected for hunting, herding or guarding tasks. On this argument, humans removed dogs' ancestors from their natural environment many thousand years ago, thus 'freeing' them from the selective pressure of natural selection (and demands for adaptation). This process produced an animal possessing artificially confused behaviour organization. They claim, therefore, that dogs should not be seen as almost human, instead, they are a purpose-bred 'soft version' of a potentially dangerous predator and any other impression of the human caregivers regarding the uniqueness of their pets is just imaginary.

In the last few decades, however, ethology has provided a somewhat different view of dogs and our relationships with them. A growing body of empirical research supports the notion that for dogs, human social environments provide their natural niche: dogs' social competence was selected and formed by humans, through developing co-operative relationships. Therefore, dogs can be viewed as not just a tamed social carnivore around us; rather, multifunctional psychological relationships may exist between people and dogs. More importantly, although ethology is often regarded as the science of non-human animals' behaviour, it also played a significant role in the development of the modern views of human attachment (Bowlby, 1969).

Attachment is a broad term, initially defined by psychologists as a lasting psychological connectedness between two individuals, typically between the mother and her infant (Bowlby, 1969). Although this may sound elusive and applicable only to human social relationships, it is not exactly true. Animal behaviourists, including traditional European ethologists like Konrad Lorenz, saw attachment as a behavioural phenomenon, defined based on objectively measurable criteria (Rajecki et al. 1978). In brief, in ethological accounts, attachment is an organizational construct belonging to a behavioural system, manifesting itself as long-lasting attraction to a particular set of stimuli, through

particular behaviours directed towards these stimuli, or 'objects of attachments' (Wickler, 1976). Moreover, attachment behaviour is always a product of maturational processes that denotes one-to-one relationship with a particular other, manifesting itself in different species-specific behaviours. We talk about attachment if the behaviour of the subject fulfils the following behavioural criteria (Rajecki et al., 1978):

1. During exploration and when experiencing danger, subjects should display specific proximity- and contact seeking behaviours towards a particular individual (object of attachment), which is at least quantitatively different from similar actions performed towards any other individuals.

2. In the absence of the object of attachment, the organism should show separation anxiety in response to environmental stresses.

3. The subject should show specific behavioural changes upon encountering the object of attachment after stressful separation ('greeting' and 'behavioural relaxation').

Therefore, attachment can be viewed as a behaviour-controlling structure, which evokes specific actions in case of stressful (e.g. separation from the object of attachment). This operational description constitutes common ground for both ethologists and psychologists in studying parent-offspring relationships or companionships of different species, including humans, chimpanzees and other mammals. This provides not only a comparative basis for our understanding of attachment in different species but provides some insight how human-animal relationships work.

Affectional ties (or affiliative behaviour) manifest in specific behaviours; the subject tends to remain close to the attachment figure, feels distress at involuntary separation from his/her partner and seek security and comfort in the relationship. Thus, attachment cannot be simplified to general preference for a companion or less fear from the familiar individual.

Attachment figures have four specific features (Ainsworth, 1991):

1. being physically near and accessible (proximity maintenance),

2. being missed when absent (separation distress),

3. being a dependable source of comfort (secure base), and

4. being sought for contact and assurance in times of emotional distress (safe haven).

It is important to note that this implies we can make a clear distinction between so called '**caregiving bonds**' and '**attachment bonds'**. In a caregiving relationship (providing sensitive and responsive care for offspring by the parents), the primary features are proximity maintenance and separation distress. In contrast, turning to the attachment figure in times of emotional distress (safe haven) and using the attachment figure as a secure base are distinctive features of an attachment bond.

The concept of attachment bond can be used to study different types of human relationships (parent-infant and adult relationships) and this is also a plausible theoretical ground of developing ways to assess attachment in dog-human relationships (Topál et al., 1998).

Clearly, pet dogs' attachment to their guardians cannot be assessed with questionnaire studies, nor can we unfold the biological/evolutionary roots of dog-human relationships by only filling in questionnaires about dog-human bonds. We stress that attachment is a behaviour organizing mechanism that is measurable by observing behaviour patterns. Most of the early studies described attachment as the result of imprinting-like processes during a sensitive period. However, applying more complex operational criteria of attachment made it possible to use standard laboratory procedures to investigate attachment behaviour patterns even with adults.

2.1.1. A specific aspect of domestication

Central to ideas of human attachment is a theory based on a young child's need to develop a relationship with at least one primary caregiver for his/her normal social and emotional development. It is an important question whether this model could be extended to the relationships of dogs and their human caregivers. While there are many possible mechanisms to achieve mutual attraction within a species, the situation is more complex if such attraction develops between dogs (or other animals) and people. Obviously, for attachment to occur between

members of different species, there must be some similar behavioural structures in both species, sharing a common function.

Domestic dogs are promising candidates for forming attachment relationships with humans. During their domestication, specific changes accumulated in the social-affiliative behaviour system of dogs (Miklósi, 2007) and these unique changes may serve as the basis of the developmental emergence of dog-human attachment. These changes are clearly shown by comparative studies of dogs and their wild ancestors. Dogs, unlike tame wolves, can develop specific preferences towards human subjects and overall dogs show stronger attraction toward humans than wolves. Although some individual and breed differences may exist in the precise timing and quality of socialization, the primary socialization period for dog puppies, during which they can establish stable affiliative relationships with humans is relatively long. Once this system of preferences and attachments has been formed these serve as a basis for later social competence.

In contrast, if somebody wants to tame wolf cubs, they need an early, intensive, and individual socialization by human caregivers, a procedure substantially different from that of the usual upbringing of dog puppies in human families. An important aspect of wolf-dog differences is that in order to achieve proper socialization, exclusive access to the desired bonding partner (human) is not necessary for dog puppies. In wolves, by contrast, exposure to conspecifics before the age of 8-10 weeks leads to a persistent fear of humans.

2.1.2 How can we measure attachment objectively?

The Ainsworth's 'Strange Situation Test (SST) was originally designed to investigate and evaluate human infant-mother attachment (Ainsworth & Wittig, 1969). We have adapted and extended it to study adult dogs' attachment behaviour towards people (Topál et al., 1998). This experimental procedure was able to provide deeper insight into the origins, development, and controlling mechanisms of the dog-human bond.

The test consists of seven episodes, each lasting 2–3 minutes, when the dog is either with the primary caregiver (owner), either with a stranger or alone in an unfamiliar place. Human participants follow detailed instructions that determine their behaviour during the test. The essential element is that separation from the attachment figure in unfamiliar environments evokes moderate stress and anxiety, shown behaviourally in proximity seeking (e.g. standing by the door), while the reunion with the caregiver evokes contact-seeking behaviours (e.g. approach, physical contact). The whole test session is videotaped and analysed later, focusing on relevant behaviours such as exploration, play, greeting, physical contact, follow, stand by the door, etc. The evaluation is based on the dog's differential reaction to the owner and the stranger. In Topál et al. (1998) study dog-owner relationships were found to be analogous to child-parent attachment behaviour because the observed behaviours were similar to those described in mother-infant interactions. The secure-base effect was revealed by the dogs' increased exploration and increased play in the presence of the owner in the unfamiliar place. When separated from the owner, dogs stood most of the time at the door even though the stranger was present, which suggests dogs' strong preference for their primary caregivers in stress situations. Moreover, dogs showed characteristic proximity and contact seeking behaviour towards the returning owner, which were different from the greeting behaviour directed at the stranger. The revealed human analogue attachment behaviour was explained by the specific effects of dog domestication.

Multivariate analysis of the data (factor and cluster analyses) separated three key aspects of dogs' behavioural structure in the SST. These major factors revealed that the dogs' behaviour during the test was affected by:

1. their sensitivity to the separation from the owner (Attachment),

2. the degree of stress the unfamiliar environment evoked from them (Anxiety), and

3. their responsiveness to the stranger (Acceptance).

The individual behaviour patterns of particular dogs could be explained with the different combinations of these determining factors.

4.2.2 Behavioural analysis – Data collection

Measured variables

Mutually exclusive variables measured during the episodes:

- play (duration)
- being by the door (duration)

Overlapping variables measured during the episodes:

- contact with stranger (duration)
- contact with owner (duration)

Behaviours measured during leaving and entering the room:

- approach (score)
- physical contact (score)
- follow (score)

Students will collect the data working in groups of 4-6 observers, using a stopwatch and the provided form. Two members of the group register the periods (one of them the owner related data, the other the stranger related data), which are reported by the other 2-4 students. Durations are measured with a stopwatch that for each measurement must be restarted so that the sequential periods could be listed one under the other on the data sheet. In case of the behaviours that are rated with scores (e.g. approach, follow) students dictate the relevant scores to their pairs.

4.2.3 Data Analysis

Students will analyze the date individually, and not in the groups. .

As the data of the two tested dogs alone are not suitable for statistical analysis, for further evaluation the mean/median values of the coded data will be merged to an existing larger database. This way - due to differences in the coding - each group will have a somewhat different dataset and results of the statistical analysis.

The steps of data analysis:

1. Calculating the mean values of the coded time periods, and using the full length of the episodes to calculate relative durations (in the case of the scores the simple calculation of means/medians)

2. Merging the calculated values with the data set of previously observed dogs (the previous results are available in an Excel file on the classroom computers).

3. Performing the statistics using the InStat program: normality test, group means calculations, paired t test. (The demonstrator actively assists in carrying out the statistical calculations.)

4. Registering the calculated values: means, standard deviations, test statistic value, degrees of freedom, significance level records.

Joint DISCUSSION of the results.

3. MATERIALS

The practical is led by a demonstrator who is experienced in conducting and assessing SST with dogs. The protocol is valid for groups of 20-30 students.

3.1 Subjects and tools

Two family dogs' behaviour will be observed in the Strange Situation Test. Based on the results of previous tests, such dogs are chosen for the practical, which will show most probably different attachment types. They have to be calm and feel comfortable in situations when many strangers are around them.

Two ball, two rugs, and two chairs are required, and eye-level high folding screens, which separate the test area from the students. A stopwatch is needed for every fourth student. Data collection is conducted using paper forms, and then the data is transferred to Excel and analysed by InStat.

4 PROCEDURE

After a short theoretical introduction the practical is divided into three sub-tasks. The first merely serves to provide students with the minimum routine in using the method. This part of the test is not live but a video footage of a previous test is projected, because this task serves only the purpose that students acquire the minimum routine required for coding the measured behaviour elements.

After this part the hypotheses and the methods of measurement and data analysis are jointly determined based on the theoretical introduction and experiences with the video records.

In the following, two shortened SST (1-minute long episodes) will be conducted, where students code the behaviour of the dog during the test. Finally, we evaluate the results together, the students work with the data of their groups, but the notebooks are to be completed independently.

4.1 Practicing the method – coding behaviour variables (Video)

Watching an SST on video, we determine the specific behaviour variables that can refer to the specific preference the dog shows toward the owner. We discuss the criteria for identifying these behaviours/contexts, and the students try out the division of labour within the group: some of them write the data told by others (the 'observers') on the data sheet. The size of the groups depends on the number of students. This way everyone will have time to pay attention to the test, but in the meantime, the most important variables are recorded. (For the same reason, we will use a simplified/shortened list of the originally coded variables.)

The purpose of this task is to master quickly the technique and learn to identify the characteristics of the behaviours typical in an attachment bond. The task will continue until each team will be successful in the coding.

4.2 Strange Situation Test

Consecutive coding of two SST on two dogs of different attachment type.

The two tests are coded and analyzed on the same way. First the previously known characteristics of the two dogs have to be discussed. Below we give an example of two different kinds of subjects.

According to questionnaire data (filled out by the owner) or previous test results:

- Dog 1 - weakly bonded, has a tendency to interact with strangers, a bit worried about unfamiliar places/situations
- Dog 2 - strongly bonded, avoids interaction with strangers, it is not nervous or unsecure in unfamiliar places/situations

4.2.1 Hypotheses and predictions

Based on the literature, which initial hypotheses can be formed?

What a priori predictions could be specify based on the selected variables?

For example:

- The duration of *standing by the door* would differ in the presence of the owner and stranger.
- Dogs will show different *greeting* behaviour towards the owner and the stranger (approach, duration of physical contact).
- The response of the dog is different when the owner or the stranger leaves the room (follow).

4.3 Preparation of the Report

The report, based on the datasheet of his/her group, must be completed by each student separately

The report shall include:

- research question
- hypotheses, predictions,
- brief description of the method,
- results
- and their short discussion.

4.4 General evaluation – Considerations for the discussion

- Have the collected data supported the hypothesis? Have any prediction been proved?
- Can the results be explained by any alternative hypothesis?
- Was the selection of variables relevant?
- What would you do differently if you had to extend this study?

Answering questions together.

Figure XVII.1 Data sheet for the Strange Situation Test

Figure XVII.1 Data sheet for the Strange Situation Test 1

DATASHEET – SST 1											
Name:……					Group: ……						
Date:……			No:……		Dog: ……						
plyO	**plyS**	**dooO**	**dooS**	**conO I**	**conS**	**appO**	**appS**	**greO**	**greS**	**folO**	**folS**
Sum	time %	mean									

Figure XVII.2 Data sheet for the Strange Situation Test 2

DATASHEET – SST 2

Name:…… Group: ……

Date:…… No:…… Dog: ……..

plyO	plyS	dooO	dooS	conO I	conS	appO	appS	greO	greS	folO	folS
Sum	time %	mean									

Figure XVII.3 Report form (two pages)

REPORT

Name: ..... Group: ..... Date: .....

Question .....

.....

Hypothesis, predictions

.....

.....

.....

.....

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Methods

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.....

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.....

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.....

.....

Result (group level) fig 17.3a

	in the presence of the OWNER		in the presence of the STRANGER	
	mean	**SD**	**mean**	**SD**
play				
door				
contact				
approach				
greeting				
follow				

Report page 2

Results of the statistical analysis (fig 17.3b)

	stat test	p value
play		
door		
contact		
approach		
greeting		
follow		

Discussion

Dog 1: .....

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Dog 2: .....

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Group level

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Chapter XVIII. Assessing the inner state of dogs based on their barks; is there difference between the nuisance level of the barks?

Péter Pongrácz

1. OBJECTIVES

During the practical the students can participate on both 'sides' of a scientific experiment. At first they are the subjects of a playback study, where they assess pre-recorded dog barks. They have to categorize the context of barks and evaluate the possible inner state of the dog based on its vocalisation. In the next part the students will analyze the collected data, working with the summarized data sheets of each participating students. The main research questions of this practice are (1) whether dog barks convey contextual (functional referential) information; and (2) whether dog barks carry emotional (non-referential) information for the humans. An additional question that we will analyze whether the dog barks can be considered as nuisance for the humans on a different level?

2. INTRODUCTION

2.1 Dog-human communication

Keeping dogs as pets is a widespread hobby in the western societies. Dogs are known as the first domesticated animals, and the co-habitation between humans and dogs has a many ten thousand year old history (Vilá et al., 1997). Although dogs participate in the everyday life of the majority of the human population, the scientific (ethological) research on dogs' behaviour became truly relevant only towards the end of the 20th century. Researchers on the Department of Ethology of the Eötvös Loránd University had a lion share in forming a new point of view on dog's behaviour: namely that the natural behavior of dogs can be understood only if we consider the effect of the humans on it. The main reason behind this hypothesis is that although the selection of dogs had and has multiple functional reasons, the natural habitat/ environment for the majority of dogs is the human group, or community.

The success of the dog-human co-existence depends on the presence of a handful of key features in the dog. Among these the most important are the **attachment** (Topál et al., 1998), which can be considered as an analogy of the attachment between human infants and their caregivers; the **capacity for (interspecific) social learning** (Pongrácz et al., 2001, 2008; Kubinyi et al., 2009); the **capacity and willingness for paying attention to humans** (Miklósi et al., 2003); and being able to utilize and understand the various forms and channels of (interspecific) **communication** (Soproni et al., 2002; Pongrácz et al., 2004; Topál et al., 2008). For example, dogs can learn easily how to understand/ follow the acoustic and visual signals of the humans, and dogs use (spontaneously) such signals toward humans, like staring, gaze alternations and making eye-contact with someone.

Members of the Canidae family possess a rich repertoire of communicative signals. It contains various **chemical** (olfactory), **acoustic** (sound) and **visual** signals. The domesticated member of this group, the dog is not an exception either. There is an intriguing question that if during the domestication dogs were selected for the capacity that made them able to learn and utilize human communication, did something similar happen to the humans, too. This would mean that humans became aware of canine communication and they are able to understand it at least partially. For finding the potential answer to this question, it is worth to consider how specific are the signals that are emitted by dogs through different channels of communication. The **chemical signals** of the dog have a multitude of meanings, including the identification of sex and individuals, reproductive status and social rank. These smell compounds are very species specific and at the other hand, the olfactory capacity of the humans may not be developed enough for the extent of differentiating among such subtle differences of another species. The **visual signals** of dogs are conveying such various information as different types of agonistic intentions (threatening, defense, sub-

mission), or the initiation and maintenance of playful interactions (Bekoff & Allen, 1998). Dogs definitely use visual signals that are easily understandable for humans (like the previously mentioned gaze alternation, eye contact and staring). However, we know about situations where the (mis)understanding of typical canine communication signals can result in fatal consequences. Meints and colleagues (2010) tested young children in an experiment, where the subjects had to categorize portraits of dogs according to the suspected emotional state of the depicted animals. Children usually mislabeled the pictures where aggressive dogs were shown with bared teeth – telling that these dogs were 'happy'. The mistake is easy to understand, as the wide pulled mouth and visible teeth mean smile in the human facial communication of emotions, but when this kind of error occurs during a real dog-child interaction, the misunderstanding can have tragic results. According to the authors, many of the dog bite accidents where the injured party was a child can happen as the consequence of an unlucky interaction, when the dog was showing its unwillingness in (further) close-contact activity with the child, who eventually misread the seemingly 'smiling' dog's signals. We can draw the conclusion that to understand the visual signals of a dog, humans may need considerable experience to overcome the differences between the two species' signal repertoire, caused by for example the anatomical divergence of humans and dogs.

2.2 The acoustic communication of dogs, with emphasis on barking

The first comparative studies were done in the 1970ies, compiling and comparing the vocal repertoire of the dog and its closest wild living relatives. Tembrock (1976) listed 14 different types of vocalisations from nine contexts when discussing the vocal communication of the dog, red fox, gray wolf and the coyote (see Table 1).

When comparing their vocal repertoires, dogs show considerable similarity to their wild relatives with a major difference. **One of the vocalisation types, barking occurs with a striking asymmetry across the species. While dogs bark in almost all the possible contexts, wolves, foxes and coyotes use this kind of signal only in agonistic situations.** A further difference between the way dogs and their wild relatives bark is the length of an average bout of barking. While wolves emit usually only a few barks at a time, dogs bark in a highly repetitive manner, and dog barking can go on continuously for several minutes.

In vain barking proved to be the most typical form of vocalisation for the dog both on the quantitative and the qualitative level; it did not receive too much scientific interest until the end of the 20th century. Probably the main problem for the ethologists was the fact that it was hard to find any communicational meaning for the dog barking. In other words, if we consider the contexts where dogs use barking (see Table 1), it is not clear how a dog would react in these contexts if it hears another dog's barking. Perhaps this was the main reason why the authors of the earlier studies did not mention any communicative role when discussing the origin and function of barking in dogs. Some scientists (Coppinger & Feinstein, 2001) hypothesized that barking is a feature of neoteny in dogs (a feature is regarded as 'neotenic' if it is found in adult individuals however it is normally a characteristics of the juvenile animals); others considered barking as the indicator of the general excitement level of the dog (Cohen & Fox, 1976); and in a recent study the authors found dogs' barking comparable to the mobbing signals of many other species (Lord et al., 2009). Although none of the previous authors denied the effect of domestication on the dogs' acoustic signals (as domestication can be regarded as perhaps the most important source of differences between the dog and its wild relatives), the common feature of these hypotheses is that they explained the peculiarities of dog barking with selective forces independent from communication. Contrary, they regarded the development of dog barking free from communicative value, regarding it rather as a product of the 'relaxed selection' during domestication.

At the beginning of the 21st century fresh theories and also empirical evidence appeared, heralding a new approach to the possible communicative function of dog barking. These hypotheses included a shift regarding the possible 'receivers' for dog barking during the domestication. According to Feddersen-Petersen (2000), when dogs joined to the human social groups, the former need for long distance acoustic communication (like the howling) started to diminish. Instead, such vocalisations were favored that conveyed information from medium to short distances – and barking was just suitable for this new role. By the explanation of Feddersen-Petersen, dogs had to communicate with not only each other, but humans appeared in their communicative sphere also. Yin (2002; Yin & McCowan, 2004) made an important discovery by proving that barks that were recorded in different contexts have distinct and consistent acoustic features. This result served as an indirect proof of that barking may still have some embedded information content. After more than a decade of research at the Department of Ethology of the ELTE we base our theory of acoustic communication in dogs on the realization that as a consequence of domestication

humans became the most important social partners for dogs. As a result, the communicative system of the dog must show some kind of an adaptation to the change in the social environment as well. Our main research hypothesis was that the qualitative and quantitative changes of dog barking (its spreading to almost each of the communicative contexts, and its repetitive proliferation) may serve the communication at least partly towards a 'new audience' – the humans. A long series of experiments has been conducted, based on the method of sound playbacks, where human listeners had to evaluate and score different dog barks, along with the acoustic analysis of the sound. Based on this empirical work we could formulate a new theory about the acoustic communication between dogs and humans.

2.3 Do humans understand dog barks?

The studies that are shown here in details were using very similar methods to those we will employ during this practical. Dog barks were collected on the field, with digital sound recording system. For reducing the unnecessary variability of the acoustical parameters caused by the anatomical and size variations among dogs, the barks were recorded from adult specimens of a single dog breed, the Mudi. Mudis are mid-sized (40-50 cm tall at the withers) Hungarian herding dogs of a lively temperament. We collected barks from approximately 50 dogs, in six typical contexts:

- "Stranger": The experimenter (male, age 23), who was the stranger for all the dogs, appeared in the garden of the owner or at the front door of his/her apartment in the absence of the owner. The experimenter asked the owner by phone to stay in another room, or at a greater distance, during the time needed for the recording. The experimenter recorded the barking of the dog during his appearance and intrusion into the garden or apartment for 2-3 minutes.
- "Fight": For dogs to perform in this situation, the trainer encourages the dog to bark aggressively and to bite the glove on the trainer's arm. We recorded the barks of the dogs during their training for 1-2 minutes.
- "Walk": The owner was asked to behave as if he/she was preparing to go for a walk with the dog. For example, the owner took the leash of the dog in her/his hand and told the dog „We are leaving now". We recorded the barks of the dogs during such situations for 1-2 minutes.
- "Alone": The owner tied the leash of the dog to a tree in a park and walked away, out of sight of the dog. The experimenter recorded the barks of the dog from a distance of 4-5 m in the absence of the owner for 3-4 minutes. The somewhat bigger distance between the experimenter and the dog was necessary in this case, to elicit the required barks more easily from the dogs.
- "Ball": The owner held a ball (or some favorite toy of the dog) at a height of approximately 1.5 m in front of the dog. The experimenter recorded the barks elicited in this situation for 1-2 minutes.
- "Play": The owner was asked to play with the dog a usual game, such as tug-of-war, chasing or wrestling. The experimenter recorded the emitted barks during this activity.

From the recordings we selected 10-20 s long sections for the playback experiments. Listeners had to assess usually three different sequences (recorded from three dogs) from each context. In each study listeners were given two tasks. First, they had to recognize the context of the barks, second, they were requested to characterize each bark with the help of five different inner states. These were: aggression, fear, despair, happiness and playfulness. The inner states were to be scored on a five-grade scale. For each of the two tasks the barks were played once. Here we summarize the results of three different papers.

One of our first, somewhat surprising result was that **adult listeners recognized the context of the barks regardless of their previous experiences with dogs**. Those who never had a dog were just as successful in this task as those who were dog owners, or even owned a Mudi. Averaging their answers from the six possible contexts, the three groups performed significantly over chance level. When we analyzed the answers separately for the six contexts, the best performance we found in the case of the 'Stranger', 'Fight' and 'Alone' situations, and the listeners recognized also the 'Play'context fairly well (Pongrácz et al., 2005).

The assessment of the dogs' **inner state** was strongly depending on the **acoustic characteristics of the barks**. During the analysis we focused on three basic acoustic parameters: the dominant frequency (**pitch**), the harmonic-to-noise ratio (**tonality**) and the interbark-intervals (**pulsing**). According to the listeners, the dog was aggressive if its bark was deep, harsh and pulsing fast. Obviously, the dog seemed to be 'not-aggressive' if the bark was high-pitched, clean and pulsed slower. Despair was characterized with high-pitched, very tonal and very slow barks. A dog was considered as playful if the bark was high-pitched and pulsed with a greater variability (Pongrácz et al., 2006). When the listeners were scoring the emotional valence of the barks, it turned out that their evaluation was

in good concordance with the so-called **structural-motivational rules of** Morton (1977). According to Morton's comparative study, based on a variety of avian and mammalian species, these animals **communicate their basic inner states with such vocalisations, which can be expected from the anatomical features of a specimen that typically manifests such an inner state**. For example, usually the mature, large, strong specimens will show aggression, and the voice of a large animal is usually deep and atonal. Subsequently, as vocalisations became the predictors of inner states during evolution, aggression in general is encoded by deep, atonal vocalisations across many species. The experiments showed also that **in dog barking, beside the tonality and pitch, the interbark intervals (pulsing) has also an important role** in discriminating inner states (or contexts). From the evolutionary aspect, dog barks became more abundant and repetitive compared to the barking of the wolves, therefore **pulsing could serve as an additional carrier of information, helping the dogs to become capable express a wider variety of emotions with a single type of vocalisations, the barking**.

Not only adult humans, but children also proved to be efficient in categorizing the contexts and evaluating the inner states of the dogs based on the barks. In a somewhat simpler task than the previously detailed experiment, we tested three age groups: six, eight and ten year old children (Pongrácz et al., 2011). Participants could choose from three contexts ('Stranger', 'Alone' and 'Play'), and they had to assess three inner states as well ('Angry', 'Fearful' and 'Happy'). The six year olds were able to recognize only one context ('Stranger') significantly over the chance level, however the children of the other two age groups showed comparably good results to the adult control group. The easiest inner state to recognize was the 'Angry' for the children, and they had the most difficulties with the 'Fearful' emotion. This study showed that earlier experiences with dogs did not influence the children's efficiency in the evaluation of dog barks. As a conclusion we can assume that humans are capable of recognizing the inner states of dogs when they hear them barking, and they are also capable of categorizing the contexts that the barking was originated from. We cannot rule out the role of learning in this process, however we hypothesize that humans recognize dog barks mostly on the basis of the Morton-rules that help us to decipher inner states of a caller in the interspecific communicative interactions.

2.4 Nuisance barking

At the time this chapter was written (March 2013), a Google-search was performed by the author with the key words "dog barking". Among the first ten hits five belonged to articles that were about how to lessen or stop 'nuisance' or 'excessive' dog barking. (From the other five hits three were video footages on YouTube, one further provided downloadable dog barks to cell phones.) This short and non-scientific survey illustrated convincingly that for the average people barking is much more one of the annoying sources of noise than information. Nuisance barking can have serious consequences when it is reported to the authorities: dog keeping can be banned or restricted in the city, or district, or in particular buildings; legal processes are also fairly common following the complaints of neighbours who found dog barking excessively annoying. Compared to the amount of cases, there is a surprising lack of professional empirical evaluations of what levels of dog barking can be considered as 'too high', or 'disturbing'. Among the few examples, in 2011 in the city of Los Angeles they defined barking as nuisance, when a dog barked for more than 10 min continuously, or for more than 30 min within three hours periods.

Stopping excessive barking represents a hard task for dog owners and dog trainers. It is not surprising that there are various devices and methods available on the commercial market for bark reduction. Assessing of the effectiveness of these is obviously not the ethologist's task. Among the antibark devices we know special muzzles and muzzle harnesses that prevent the dog in opening its mouth. The other type of these devices includes such collars that have sound sensors and if the dog starts to bark, the collar blows citronella vapor or punishes the dog with a mild electric shock. As a terminal solution, many owners opt for the so-called de-barking operation at the veterinarian, which means that the vocal cords are rendered useless by surgical intervention.

There are no scientific data about which barks represent stronger nuisance sources for the humans. During this practical the students can evaluate bark playbacks from several individual dogs, recorded in different contexts. Our question is whether some of the contexts elicit more annoying barks from the dogs than the others. A similar study on a representative sample of people would be useful both for the dog owners and for the official authorities to understand the circumstances and prevent the emission of nuisance barking.

3. MATERIALS AND METHODS

3.1 Location

The practice is conducted at the Department of Ethology. Students prepare the practical reports later from the collected data, then they submit the reports before the pre-set deadline.

3.2 Subjects

The students themselves act as research subjects, because they will assess the pre-recorded barks. After the bark playbacks they score the vocalisations' emotional content and the nuisance scale, and in a second test they try to recognize the context of the barks. Each student will have an own scoring sheet, and the statistical analyses will be conducted on the summarized data of the whole group.

3.3 Materials

Students will use the scoring sheets we provide for them at the Department (see the sample sheets at the end of the chapter). During the practical, everybody has to assess 18 individual bark recordings. Each bark was recorded from Mudi dogs. The barks belong to six contexts, and from each context we play back three different samples (recorded from three different dogs). Each bark sequence is approximately 10 s long.

4. PROCEDURE

4.1 Recognition of the context

The demonstrator plays back the barks one by one, keeping long enough break between two barks so the students can mark their guess about the context. The six contexts are known (Stranger, Fight, Alone, Walk, Ball, Play), from which the barks were recorded. At the end of the practice the demonstrator unveils the real contexts of the barks to the students.

4.2 Scoring of the inner states and the nuisance level

Performing this task the barks are played back again one by one. Each bark has to be scored on each of the five scales of inner states (aggression, fear, despair, playfulness, happiness). Additionally, the barks have to be evaluated according to their nuisance level. Scoring is done in each case with the help of a 100 mm long line on the scoring sheet. Students put a mark on this line, based on how strongly a given inner state characterizes the barking dog by their opinion. The left end of the line (0 mm) marks the "not at all" value, and the right end of the line (100 mm) means "extremely". The nuisance level of a given bark is evaluated the same way. After the practice students have to measure the distance of each mark from the left end of the line, and these measurements yield the inner state and nuisance data.

4. 3 Data analysis and presentation of the results

4.3.1 Summarizing the individually collected data

The results are calculated from the summarized data sheets. The demonstrator provides a previously created Google Document for the students, where everybody can enter his or her individually collected data. It is important that the uploading of the data should happen within the deadline, because after the deadline the demonstrator closes the editing of the document, therefore each student can start the data analysis from the same summarized data sheet.

4.3.2 Context recognition

The goal of this analysis is to find out how accurately the students guessed the context of the barks. Parallel with this we can see if there were typical trends for making errors during the context recognition. There are several methods of how to visualize (illustrate) the results, however, one of these is a mandatory requirement for the practical reports: this is the so-called **confusion matrix**. One side (vertical or horizontal) of the matrix contains the guessed contexts, and the other side contains the real (correct) contexts. Students have to express the ratio of guesses in percentages. For this, an average should be calculated within each context from the answers (guesses) given to each of the three barks. Here is an example how to do it: let's say that 20 students participated on the practice. This means that each bark received 20 guesses about its context. We have to count how many guesses were given for each context in the case of the first (second, third…) bark. If we express these numbers in percentages, the total of the six contexts should be 100. When we calculated the percentages for each of the three barks within a particular context, we calculate of their averages in the case of each context. Obviously, the sum of these averages should be 100 again. This process should be repeated for each context. From the average percentage values we can build up the matrix (see Figure 1). The ratio of the correctly guessed contexts will be shown along the left-right diagonal of the matrix. Ratios of the erroneously guessed contexts will be placed in the other cells. It is recommended to apply colour or tone shading for the cells, according to the ratio levels.

The results should be discussed on the basis of the confusion matrix. Which were the most and less successfully recognized contexts and possibly why? It is worth to remember that when there are six possible answers, the chance level is 16.7% for each context, so the actual ratio of the correct guesses should be compared to this chance value (16.7) during the analysis. Students should discuss the most obvious trends of typical contextual misidentifications. What can be the explanation for particular contexts are often confused, while other pairs of contexts are hardly ever confused with each other?

		Real contexts					
		stranger	fight	alone	walk	ball	play
	stranger	24%	26%	28%	2%	20%	1%
	fight	31%	46%	13%	1%	4%	10%
	alone	12%	0%	32%	28%	19%	0%
	walk	14%	7%	9%	42%	18%	11%
	ball	11%	8%	14%	15%	25%	22%
	play	7%	12%	4%	12%	14%	57%

Figure XVIII.1: Example for a confusion matrix. Along the diagonal of the matrix the ratios of the correctly recognised contexts are presented.

4.3.3 Results of the emotional scoring

The main goal of this analysis is to see, which inner states were thought to be the most characteristic for each context of barks. In other words, how did the students evaluate the inner state of the dogs based on their barks? On a similar way it can be also discussed, which barks were the most and the least annoying for the listeners.

Each bark sample has been given five scores (between 0 and 100 mm) along the emotional scales, plus an additional score on the nuisance scale. At first we calculate the average score given to each bark separately for the inner states and the nuisance level. After this we calculate the average inner state and nuisance scores for each context from

the values of the three barks belonging to the same context. Results should be illustrated with charts. A possible option for such illustrations is shown by Figure XVIII.2.

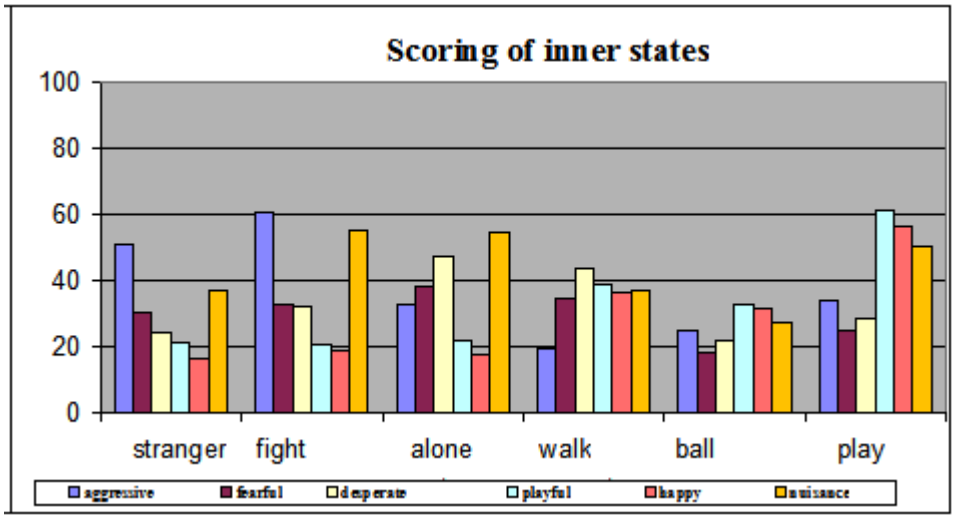

Figure XVIII.2: A possible way how to illustrate the results of the emotional scoring. It is important that the scale on the vertical axis should extend to 100.

When discussing the results, students should explain which inner states were thought to be the most and least characteristic for particular contexts, and what kind of explanations can be found for these. We should keep it in mind that there are no 'correct' and 'wrong' answers in the case of the emotional scoring, because as we did not measure originally the inner states of the barking dogs, the correctness of the emotional scoring cannot be validated directly.

We should discuss the possible reasons of the results regarding the nuisance level of the particular contexts. Besides the averaged values, it is worth to take a look on the results of the individual bark samples, too. It is possible that not the given context, but the vocalisations of a particular dog is proven as the most annoying for the listeners.

4.4. Evaluation of the practical report

The work of a student is considered eligible, if (1) he/she submitted his/her individual data for the summarized data sheet, compiling the formal and content requirements; and (2) the student wrote and submitted the practice report that contains among others the result of the data analysis. We evaluate the practice report based on the following standards:

- Did the student write a detailed introduction, including the scientific background of the research, the experimental question and hypotheses?
- Did the student explain the methods and materials of the experiment?
- Were the necessary statistical analyses performed and presented in the report?
- Were the results illustrated with acceptable graphs/ figures?
- Did the student explained and discussed the details of the results?
- Were the mathematical formulas and statistical analyses correct?
- Does the report include a general discussion, where the student draws the broader conclusions of the study, and connects the new results to the former knowledge based on the literature?
- Does the report fit to the formal and aesthetical requirements?

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	meow	grunt	whine	yelp	scream	yip	howl	coo	growl	cough	bark	snort	toth snap	pant
Greeting	F	WD	R C D	D	F	C	WD	F	WD	-	D	-	-	R
Play initiation	-	-	D	D	-	-	-	-	-	-	D	-	WD	RK
Submission	F	-	W C D	D	W C D	-	-	-	-	-	-	-	-	-
Defense	-	-	W C D	D	CF W	-	W	-	W C R D	W C R D	W D	F	W C D	-
Threatening	-	-	-	-	-	-	-	-	W C R D	W C R D	W C R D	F	W C D	-
Contact seeking	nF	D W	n, D W C	n-W n-C D	F	C	-	F	-	-	D	-	-	-
Pain	n	-	n, D W C F	n-W n-C D	n, D W C F	-	-	-	D	-	D	-	-	-
Loneliness	-	-	n, D W C	D	-	-	W C D	F	-	-	D	-	-	-
Group	-	-	n, D W C	-	-	C	W C D	-	W C D	-	-	-	-	-

Table 1: Contexts and types of vocalizations of the dog and three related canid predators (based on Cohen & Fox, 1976). Abbreviations: D= dog; W= wolf; C= coyote; F= fox; n= newborn.

SCORING SHEET FOR DOG BARK PLAYBACKS – 1 (CONTEXT)

Date:

Name of the participant:

Experience with dogs: Have a dog/ Had a dog/ Never had a dog

Dog(s) in the neighbourhood: yes/ there was earlier/ never

Bark sample	context
1.	
2.	
3.	
4.	
5.	
6.	
7.	
8.	
9.	
10.	
11.	

12.	
13.	
14.	
15.	
16.	
17.	
18.	

SCORING SHEET FOR BARK PLAYBACKS – 2 (INNER STATE)

Bark sample	**Inner state**	
1.	Aggressive	
	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
2.	Aggressive	
	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
3.	Aggressive	
	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
4.	Aggressive	
	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
5.	Aggressive	
	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
6.	Aggressive	

	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
7.	Aggressive	
	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
8.	Aggressive	
	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
9.	Aggressive	
	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
10.	Aggressive	
	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
11.	Aggressive	
	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
12.	Aggressive	
	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
13.	Aggressive	

	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
14.	Aggressive	
	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
15.	Aggressive	
	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
16.	Aggressive	
	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
17.	Aggressive	
	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	
18.	Aggressive	
	Fearful	
	Desperate	
	Playful	
	Happy	
	How annoying?	

Chapter XIX. Localisation of animals by radiotelemetry

Vilmos Altbäcker

1. OBJECTIVES

The aim of this practical is to introduce students to a technique widely used in field biology including ethology. Since many wildlife species are elusive and difficult to observe, radiotelemetry has provided an invaluable tool to learn more about their secret lives. Despite its popularity, one should also consider its limits, as radio-telemetry may turn out as inappropriate under many circumstances. It is an expensive and time-consuming technique. Despite the frequency with which radio ransmitters are attached to research animals, surprisingly little is known about their effects on the behaviour of the target species. The present practical is an introduction to the pros and cons of this technique used for surveying habitat use, home range, movement pattern, and demographic studies in field studies.

2. INTRODUCTION

Radio telemetry is the transmission of information from a transmitter, attached onto a free-ranging wild animal, to a receiver. It is also known as radio tagging or radio-tracking. Advances in the field of wildlife telemetry have made it possible to acquire detailed data on many aspects of field biology, including habitat use, home range size, mortality, survival, and migration. As a result, radio-telemetry became a widespread tool in field biology.

This introduction is organized into several sections reflecting the successive steps required to plan and conduct a study involving radio-telemetry.

1. In the first section, we discuss the steps required to initiate a telemetry study and the humane treatment of the studied animals.

2. The second and third sections deal with the technical background. Basic information is presented about the mechanics of radio transmitters, and how they are safely attached.

3. The fourth and fifth sections focus on signal reception. Information is presented about options for receivers and antennas, as well, as recommendations for successful localisation.

4. In the sixth section, the design of radio-telemetry studies is discussed in relation to specific objectives. General considerations are presented for studying habitat use, home range, movement pattern, and demographic studies.

It is recommended that experienced researchers be consulted for advice, particularly for first time studies in new areas or with unfamiliar equipment. Technical problems arising in virtually all telemetry projects are often not discussed in the literature. Consultation with experienced researchers is recommended to prevent such problems.

Ethical Considerations

Prior veterinary review is strongly recommended for all studies involving radiotelemetry. In addition, experienced reviewers and vendors can provide valuable guidance regarding transmitter weight, attachment method and capture protocol, this helps to avoid problems which have already been solved by other professionals.

Because of the invasive nature of telemetry projects, researchers should be particularly aware that proper field procedures are followed. Apart from the obvious humane considerations, streesed animals influenced by the capture technique and/or radio tag itself will not behave normally.

Researchers planning a radio-telemetry study should encertain that study animals are captured humanely and the transmitter attachment produces only minimal disturbance later. Capture should be performed with minimal stress to the animals by optimizing its timing to avoid disturbance of animals when they are breeding or raising young.

If anaesthesia is required, it should only be performed by trained personnel and the animal should not be released or left unattended until it is completely recovered.

Transmitters must be attached with minimal side effects to the study animal. Researchers should take extreme care when fitting harnesses and collars to ensure that they allow free movement, but are tight enough to prevent them from being lost. The best method is to test attachment methods on captive animals that allows novices to practice the handling of animals and transmitter attachment under controllable conditions. Zoos are especially suitable to test transmitter attachments.

Studies performed in public areas such as in parks should also consider public opinion during the telemetry study as some people are especially sensitive to the sight of wildlife being caught and later moving around with collars. Small ear tag transmitters may be suitable for this type of location, and drop-off collars/ harnesses or implanted transmitters are to be used if possible.

Transmitters

Conventional transmitters consist of an antenna, a power source and a transmitter unit. Although this combination is fairly fundamental, the specific components chosen may vary between projects. In light of this, rather than attempt to recommend a particular type of transmitter, it is likely more useful to the researcher to describe the basic equipment options which are currently available for transmitters.

Power sources

The battery capacity, operational life and duty cycle requirements determine the radio frequency energy the transmitter circuitry can generate and deliver to the antenna (Beaty 1990). The larger the battery capacity, and the lower the current drain, the longer the operational life of the radio transmitter.

Discussion of transmitter range tends to focus on “Line of sight” (LOS) range. This is the maximum unobstructed distance between transmitter and receiver which produces an adequate signal. Range may be influenced by environmental conditions and geographic factors. High humidity, thick fog, heavy rain, wet snow, and intervening vegetation will absorb energy from the signal. Radio waves reflecting from rock outcrops or water bodies will also reduce the signal’s energy due to phase cancellation (Beaty 1990). Increasing the transmitter power output by four times results in a doubling in LOS range, but causes fourfold decline in battery life. In general, battery life and signal range is inversely related. Applying larger batteries increases the weight of the equipment, the useful operational time, and range.

Tags (collars)

Transmitters (tags) are available as complete units (including attachment options such as collars) or as components which are assembled and finished by the researcher. Manufacturers generally package transmitter units in a metal can and/or cover them in an acrylic or epoxy resin coating to protect them from the elements (e.g., salt water) and from being damaged by the teeth, beak or claws of the animal. The transmitter circuit is usually switched off via a little magnet attached to the outside, this prevents unnecessary power consumption. Spare transmitters should be stored on a wooden shelf with at least 2.5 cm distance between magnets on different collars to ensure that the magnets do not cancel one another out and activate the transmitters (Decker 1988). A receiver should be used to check that all magnets are in place and all transmitters are turned off. Small transmitter tester units are also available at several manufacturers.

A detailed record should be kept of each transmitter unit (including those in storage) giving purchase dates, storage times, testing and results. If the transmitter fails, the log gives hints to prevent or exclude further failures.

Dead transmitters may be refurbished by replacing the battery. Proper care and maintenance of transmitters is critical to reduce the total costs of field studies.

Global Positioning System Transmitters

A GPS (Global Positioning System) transmitter locates itself by receiving and triangulating signals from at least 3 of 26 possible satellites, and then transmits its (the animal’s) position to the user. The accuracy of GPS location systems is within a few meters, but it may vary with the density of the forest canopy (Rempel et al. 1995). GPS transmitters can be also programmed to compile location data for a specified length of time, then transmit all of

the data at once when contacted by a special receiver operated by the user. In this way, several weeks of location data can be recovered during a single relocation event. The size of GPS transmitters is shrinking, while the best unit in 1998 weighed 1800g and its use was limited to larger animals such as wolves and moose, the 2013 units weigh only 30g and can be used for medium sized mammals or even raptors.

Temperature and light sensors

Temperature sensors may be used to monitor either the animal's body temperature or the environmental temperature. Body temperature data may be useful in determining health or reproductive status, and ambient temperature may also be utilized for habitat selection or hibernation studies. Transmitters for body temperature may be placed subcutaneously, internally, within the inner ear, cloacally, or vaginally (Burger 1989). Transmitters for ambient or den temperature may be placed on a regular collar or harness. Size or weight limitations and the data precision required will also affect transmitter type and placement.

Temperature data are transmitted via Pulse Interval Modulation, called PIM. The relationship between temperature and pulse rate must be carefully calibrated. Due to aging, transmitters should be recalibrated at the end of the study to correct for this error. Temperature sensing transmitters may also be used to detect mortality of warm bodied animals. Pulse rates of light level indicator transmitters are controlled by a light sensor mounted within the transmitter. This allows researchers to calculate the amount of time the target animal has spent under cover or in a burrow.

2.1 Transmitter attachment

There are many different ways to physically attach transmitters to wildlife. Strong species such as grizzly bears require very sturdily-built equipment. Even though a small mammal like a rabbit itself may not damage the transmitter, its predators capturing the rabbit may destroy the transmitter so that the researcher may be unable to locate it. The best attachment option for a particular study must be chosen on the basis of the body type, shape, size and lifestyle of the study species and the type of data required by the researcher.

Figure XIX.1. main parts of the radiotelemetric equipment: a/ radio collar attached to the animal, b/. reciever with attached Yagi antenna

Researchers are strongly urged to adhere to the following recommendations planning wildlife telemetry (after Bertram 1980 and White & Garrott 1990):

1. Always carry extra collars to replace a faulty tag or to test the receiver.

2. Also record the signal pulse rate to detect any signs of battery failure.

3. When studying group living animals, attach tags on several animals per group, this gives you extra possibilities if one radio fails.

4. Treat all animals with causing the lowest possible stress to obtain realistic data.

5. Use the smallest possible transmitter package. No tag should be heavier than 5% of the animal's body weight. For flying animals, 3% may be a more appropriate proportion.

6. Transmitter packages placed on criptic animals should be as inconspicuous as possible.

7. Transmitters and their attachments should be tried out on captive animals before they are tested on free-ranging animals.

8. Transmitters should be tested both before and after the attachment to guarantee that they are still working.

9. Allow at least one week for newly tagged animals to get used to a transmitter before collecting data, but you should also follow the animals during this period to prevent their loss due to emigration.

10. Whenever it is possible, avoid instrumenting animals during their reproductive period, as many species appear to be particularly sensitive to disturbance at this time.

11. Seriously reconsider placing a transmitter on any animal that appears to be in poor body condition or impaired in some other way, unless it is particularly meaningful to the study to follow that specific individual. Recaptured animals showing adverse effects from transmitters should not be retagged. Researchers should not sacrifice the individual for the sake of a larger sample size.

Once transmitter attachment is complete, the animal should be carefully observed before release. Short-term behaviours such as scratching at the collar or attempting to shake off a tag will generally cease when the animal becomes accustomed to carrying the transmitter. These behaviours should be distinguished from more serious effects such as improper balance, impeded movement or shifting harnesses which will require intervention. It is an unfortunate reality that many of these problems and behaviours will not be apparent or manifest until the animal is actually released and is difficult to recapture. This only serves to emphasize the importance of thorough research, preparation and testing beforehand.

Where appropriate, it is recommended to mark the collars and harnesses in order to enhance their visibility. Paint or non-metallic reflective materials may be sewn or glued to collars and harnesses; however, this is likely not appropriate for cryptic species. Metallic tape or foils should not be used as they will detune the transmitting antenna. Adhesive tapes should also not be used as they are not very durable and may foul fur or feathers. For game species or urban studies it may also be helpful to mark a contact phone number on the collar. Colour-coded collars are also available from telemetry equipment manufacturers.

Implantable Transmitters

Implantable transmitters are best suited for species in which the necks are not well-defined (e.g., snakes), or in which the head is smaller than the neck (e.g., male polar bears). It is also recommended for burrowing animals (e.g., ground squirrels).

They are also used for certain biotelemetry applications (e.g., measuring body temperature). Implants are sealed with neutral (biologically inert) epoxy, resin, or wax, and implanted into the body cavity or under the skin. The antenna may be left external to the body, implanted under the skin or it may be contained entirely within the implant unit.

Despite the initially invasive nature of this technique, one of the key advantages of implants is that they may be much less irritating (if implanted correctly) to the animal than an external tag. Implanted transmitters have a fairly limited range. Those with an implanted antenna will have an even shorter range, but will be less subject to damage or infection than transmitters with external antennas. Transmitters are also expensive to implant as they generally require that researchers employ a qualified veterinarian.

2.2 Receivers

The function of a receiver is to read the signal picked up by the connected antenna. It amplifies the signal and makes it audible to the user. Receivers are available in a variety of sizes, weights and prices from a number of national and international suppliers. Study needs will determine whether data collection is best done manually by field personnel or whether an automated receiving station should be set up. Receivers are powered by replaceable and/or rechargeable batteries, and may also be equipped with a cigarette-lighter adapter for connecting to a vehicle's electrical system or solar panel. Some models are equipped with scanners which may be programmed to switch between a number of different frequencies; this is ideal for studies with a number of animals which tend to wander. Data loggers may also be incorporated into a receiving system, and are particularly useful for automated receiving stations.

Receivers may be damaged by static electricity from clothing or car seats and by radiated power from voice communication systems (Crow 1988). To prevent this damage, clothing and vehicle seats should be treated with anti-static fabric softener, and receivers should be turned off and the antenna disconnected when getting in and out of vehicles. It is also worthwhile to note that receivers are sensitive to moisture. This is an important consideration when try to locate animals in the rain.

It can be useful to adjust a receiver up or down in order to identify the best or most functional frequency for a given transmitter. It is not uncommon for a transmitter's best frequency to be slightly different from the one identified by the manufacturer. As well, a transmitter's frequencies may drift slightly.

2.3 Recieving antenna

A specific antenna attached to the receiver is alsp necessary to obtain signals from the transmitter. Such an antenna may be hand-held or mounted on a vehicle roof, aircraft or boat. A Yagi antenna is a directional 'gain' type antenna which uses a number of parallel directors in front of the 'driven' element (the one connected to the coaxial cable) and a reflector behind the driven element in a defined mathematical relationship (Jones 1990). Directional antennae such as a Yagis or an 'H' antenna concentrate the radiated energy to the front of the antenna. Minor lobes to the sides and rear are also produced.

Antenna beam width refers to the radial distance between the angles at which an antenna is held in which an audible signal is received (the 'directionality' of the antenna). The greater is the number of elements, the smaller the beam width. For example, a 3-element Yagi antenna has a beam width of 60o in the horizontal orientation, and a 2-element H antenna has a beamwidth of 100 o in the horizontal orientation. Both antennas have wider beam widths in the vertical orientation (Burger 1991).

2.4 Localization of the collared animals - Accuracy of locations

The accuracy of a radio-location varies with habitat type and may result in biased estimates of habitat use. A common source of error is signal bounce. Signal bounce occurs most frequently in mountainous terrain where a signal is deflected by a mountain, resulting in potential errors of many kilometres. The most effective way to overcome signal bounce during ground tracking is to take many bearings from several different places. When all signals appear to be coming from the same point then there is a good chance that the animal has been located correctly. However, if the signals are coming from a number of different points then signal bounce is likely still occurring (White and Garrott 1990).

2.5 Direct localization versus triangulation

Visual observations of radio-located animals provide the best confirmation of the accuracy of the relocation data. For large animals, a reasonable proportion of locations should be confirmed by direct visual observations (some biologists use >30% as a general rule). In new study areas or with species which cannot be observed on a regular basis, it is strongly recommended that triangulation be used with an assessment of aerial fixes made using collars placed in known locations. Such trials can test the consistency and accuracy of triangulation using various personnel and methods under various environmental conditions. Results of the trials can be used to identify problems (e.g., signal bounce) and ensure that methods are adjusted to obtain reliably accurate radio locations.

When locating animals in the field, users judge the angle over which the signal sounds loudest, determine a bearing by mentally bisecting that angle, and follow the bearing to move closer to the signal. The process is repeated until the animal can be seen or its location can be fixed. This can be accomplished by circling the signal to determine a bounded area, in which the focal animal must occur,

Alternatively, if the researcher wishes to avoid disturbing the animal, or if locations must be determined at night, the process of triangulation is followed. This requires finding the intersection of several bearings. Actual location is within an error polygon around the point estimated. The size and shape of the error polygon is determined by:

1. the accuracy of the directional antennae;

2. the distance between the two receiving points;

3. the distance of the transmitter from the receiving points;

and

4. the angle of the transmitter from the receiving points.

The most accurate estimate of an animal's location is obtained by receiving fixes that are closest to the animal and at 90o from each other. To reduce the size of the error polygon, three bearings should be taken and the animal's location is estimated from the centre of the intersections. The error polygon formed by three radio bearing lines should be small enough to accurately place the animal in a single habitat polygon.

Triangulation of animals which are moving will produce even large polygons (less accurate locations). For this reason, it is difficult to accurately determine locations of fast-moving nocturnal wildlife. If triangulation is used to determine wildlife positions, error measures should be calculated and reported along with the study results. Saltz (1994) provide a useful summary of how telemetry error should be calculated, while White and Garrott (1990) give a detailed description of the methodology.

Figure XIX.2 localization of tagged animal by triangulation. Directions of strongest signals from known points (A-D) are plotted on the map and their intersection determines location of the animal.

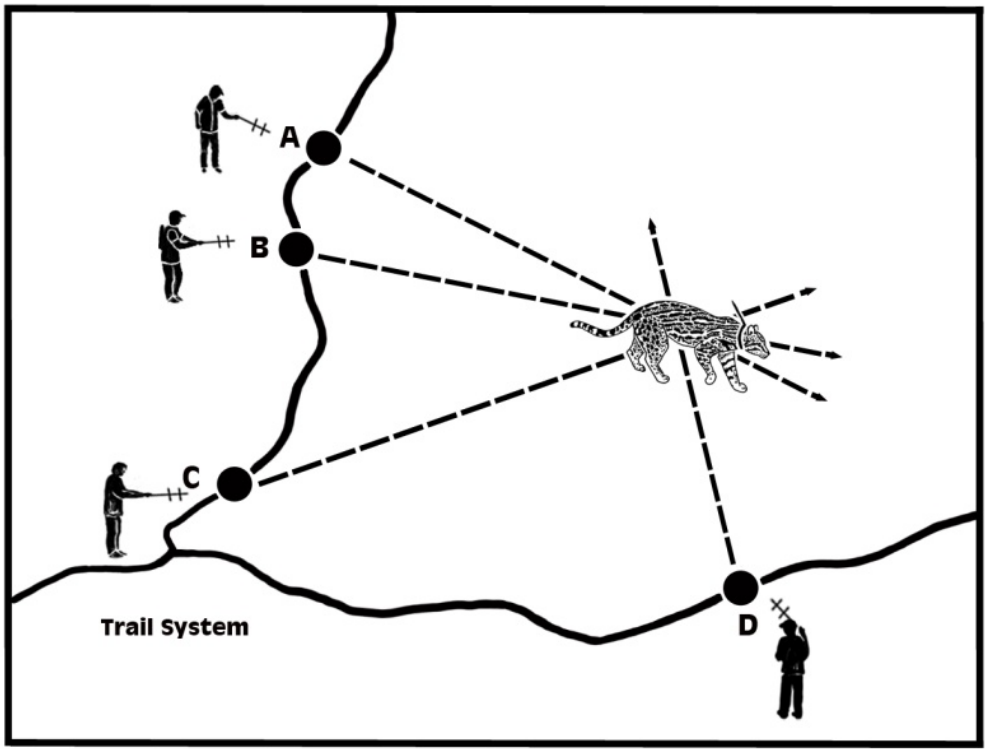
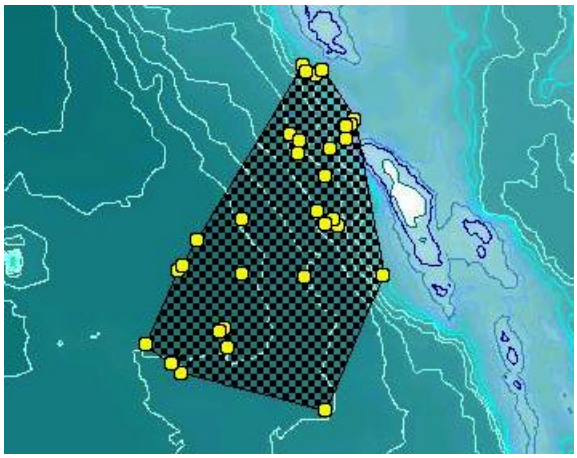


Figure XIX.2 Minimal 30 independent localizations are used to plot the minimal convex polygon as an estimate of the home range of the animal.



3. METHODS

As an introduction to basic steps in radio telemetry, we will compare the two main methods, the direct localization and the triangulation, during the determination of the location of 5 hidden radio collars.

Both methods have advantages and disadvantages. Direct localization helps to find the target individual accurately, but is more disturbing for the rest of the population. Triangulation from fixed, remote stations is less accurate (see error polygon), but can be easily automated and takes less time per individual to obtain the localisation points.

We will determine the localization of 5 collars during the practice. As variables of the methodology, we will measure the time necessary to obtain the data with both method, and the accuracy measures as the distance of data points between their mark on the map and the original location given by the teacher.

Figure XIX.4 (below) provides a Data sheet for the radiotelemetry study. After filling the data the two methods should be compared by using the Student t test in the InStat program.

DATA SHEET .....FOR THE .RADIOTELEMETRY PRACTICAL

DATE:		**EXPERIMENTER:**		
ROUP	**collar freq MHz**	**time,s**	**error,m**	**Remark**
1 direct search	**148.....**	**.....**	**.....**	**.....**
1	**148.....**	**.....**	**.....**	**.....**
1	**148.....**	**.....**	**.....**	**.....**
1	**148.....**	**.....**	**.....**	**.....**
1	**148.....**	**.....**	**.....**	**.....**
2 triangulation				
2	**148.....**	**.....**	**.....**	**.....**
2	**148.....**	**.....**	**.....**	**.....**
2	**148.....**	**.....**	**.....**	**.....**
2	**148.....**	**.....**	**.....**	**.....**
Mean direct:		**.....**	**.....**	
Mean triang:		**.....**	**.....**	
Stdev direct:		**.....**	**.....**	
Stdev triang:		**.....**	**.....**	
STATISTICS:		**t(....)=.....**	**t(....)=.....**	
		P=.....	**P=.....**	

Discussion points should include the evaluation of accuracy and workload (time) of each method as well as general considerations of applying radiotelemetry.

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Chapter XX. Methods to collect and analyse animal behaviour data

András Kosztolányi

1. OBJECTIVES

During the practical the students will get acquainted with the bases of measuring behaviour. The following topics will be discussed: Asking scientific questions. The independence of samples. How can be behaviour measured: variable types, methods of data recording, tools for data recording. Reliability and validity of measurements. Descriptive statistics and testing statistical hypotheses, simple statistical tests. On the practical we will use the topics mentioned to analyse video recordings from earlier experiments.

2. INTRODUCTION

2.1 The way of investigating animal behaviour

The collection of scientifically evaluable data has to be planned accurately. All scientific data collections start with **raising a question**. Pilot studies, previous knowledge and literature data can help us to raise an adequate question. Our goal is to formulate a scientific hypothesis and make predictions from this hypothesis. These **predictions** are specific statements that can be tested statistically (Précsényi et al., 2000).

The **measured variables** that will be used to test the predictions have to be defined accurately before data collection. This definition has to be applied consequently during data collection (Martin and Bateson, 1993). Determining of the variables is not always a straightforward or easy process. It is easy to define the body mass and its measurement, but the situation is more difficult if we intend to measure a behaviour that includes complicated, variable components such as fight or courtship between individuals. In such cases it is not always obvious when a given behaviour starts and ends, and what is its intensity etc.

The behaviour of animals is characterized by natural variability. This variability is the result of several factors: genetic factors, biotic and abiotic environmental effects and their interactions shape the behaviour of individuals (Székely et al., 2010). Because of this variability, our measurements contain 'noise' that cannot be controlled for. Therefore, to collect statistically evaluable data, several measurements have to be taken. During data collection we have to pay outmost attention to random sampling (Zar, 2010): from the group of individuals to be investigated (**statistical population**, not necessarily identical with the **biological population**) any individuals should have the same chance to be measured (**statistical sample**). If random sampling (i.e. the temporal and spatial independence of the sample elements) is not assured during data collection, then the **data will be pseudoreplicated**, and the conclusions drawn from the analysis of data may be incorrect. It is easy to see that by measuring the height of the same person twice we do not obtain two independent data points, however, assuring spatial independence is not always so simple (e.g. within a group the more similar individuals may be more close together than more dissimilar individuals). Furthermore, measurements of relatives (e.g. siblings) are also not independent, because firstly the relatives share common genes, and secondly they may developed in the same social environment.

2.2 Types of behavioural variables

Variables describing behaviour can usually be divided in **four categories** (Fig. 20.1, Martin and Bateson, 1993). **Latency variables** measure the time from the beginning of sampling until the occurrence of the behaviour. The occurrence or **frequency variables** measure the occurrence or the number of occurrences of the behaviour during a unit time, e.g. a minute. **Duration variables** measure the length of the occurrence of the behaviour. If the behaviour occurs several times during the data recording, then total duration and average duration can be calculated for the full sample. If not only the occurrence but also the extent of the behaviour (volume of a call, speed of running) has to be described, then we use **intensity variables**.

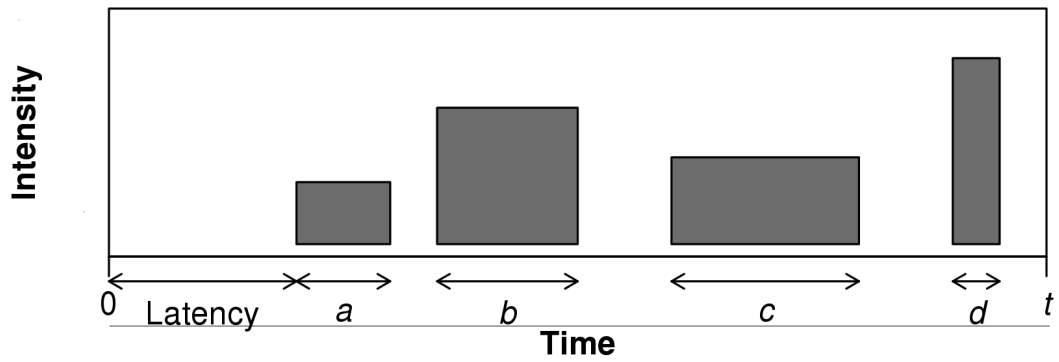


Fig. 20.1. Latency, frequency, duration and intensity. The grey rectangles represent the occurrence of the behaviour over time t. The width of the rectangles is the length of each occurrence, whereas the height is the intensity of the behaviour. The frequency of the behaviour over time t is four. The total duration is a + b + c + d, and the average duration is (a + b + c + d)/4. Based on Martin and Bateson (1993).

Before data collection, we also have to decide on which scale will each variable be measured (Figure XX.2), because the scale of measurement largely influences which statistical procedures can be used to analyse the collected data.

Scale	Definition	Example
Nominal	Categorical variable, its values cannot be ranked	gender (male, female)
Ordinal	Qualitative variable, its values can be ranked	aggression (weak, moderate,strong intensity)
Interval	The values of the variable can be ranked, and the differences of the values show the distances between the values. This scale does not have a true zero point.	temperature (e.g..40 °C - 20 °C = 20 °C, but 40 °C / 20 °C ≠ 2)
Ratio	The differences of the values show the distances between the values and also the ratio of values is defined, as the variable has a true zero point	height

Figure XX.2. Types of variables according to their scale of measurement.

2.3 Methods to record data

Behaviour can be recorded **continuously**, or only at given time intervals (e.g. every ten seconds, **instantaneous sampling**). While continuous data recording can describe behaviour very precisely, it can be used only to record a few variables simultaneously. By increasing the number of recorded variables the accuracy of continuous data recording decreases, therefore in these cases better to use instantaneous sampling. During instantaneous sampling, by the help of a stop watch or rather a timer (a device giving a short beep at given time intervals) we record at given time intervals which behaviour occurs at the sampling points. The accuracy of instantaneous sampling is largely influenced by the sampling interval, i.e. the time elapsed between sampling points. In case of swiftly changing behaviours (e.g. fight between individuals) rather short, few second intervals have to be used, whereas it may be enough to record the behaviour of resting individuals only at every minute.

2.4 Tools for data recoding

The simplest way to record behaviour is to use **paper and pencil** or pen. To make continuous data recording even an empty sheet of paper may be appropriate, whereas for instantaneous sampling usually a behavioural sheet prepared

beforehand is used. The header of the **behavioural sheet** contains the name of the observer, the date, the start and end of data recording, the identification of the observed individual(s) (e.g. name, ring number), and further data (e.g. temperature). The behavioural sheet itself is a table which rows are the sampling points, and the columns are either different behavioural variables (feeding, preening etc.) or different individuals (male, female, offspring 1, offspring 2 etc.). If the columns are behavioural variables, then at each sampling point we can indicate which behaviour occurs by writing e.g. an X in the corresponding column. Whereas if the columns represent individuals, then we can indicate the behaviour of the different individuals using one or two letter abbreviations defined previously. The biggest advantage of recording behaviour using paper and pencil is that they can be used almost everywhere any time, and there is no chance for technical failure. In contrary, the disadvantage of this recording method is that before analyses the data have to be entered to spreadsheet or database that may be a time demanding process. Entering data into a database can be avoided by using **event recorder**. Any kind of portable **computer** (smartphone, tablet, laptop) can be used as event recorder. Running an appropriate application we can record which behaviour occurs by hitting predefined key combinations or by touching the appropriate part of the screen. With an event recorder we can effectively record behaviours that consists of well defined behavioural categories, however, it may be much more difficult to add comments to the sampling points than to write down a quick note on the margin of the behavioural sheet.

The behaviour may be recorded on **video tapes**, however, that method again needs later a time consuming coding of data into a database. Video recordings have the advantage that if later during the study new questions arise, then further, **previously not planned variables** can be recorded by re-watching the footages. The disadvantage of video recordings is that on footages one can see often less than in real time, thus some details of the behaviour may not be visible. This is especially true in case of time-lapse videos where only one or a few pictures are taken per second e.g. because of limited data storage.

Behavioural data can be also recorded by **automatic devices**. For example, **electronic scale** can be placed under the nest of birds to describe the parents feeding activity based on the body mass differences of the sexes (Szép et al., 1995). Another possibility is to glue small RFID (Radio Frequency IDentification) tags (transponders) to the birds, and record the unique identification codes of tags by a computer controlled reader connected to an antenna applied under the nest or to the entrance of the nestbox (Kosztolányi and Székely, 2002). The advantage of using automatic recording systems is that big amount of data (even data from several days) can be collected and the data is directly recorded into a logger, so there is no need for time consuming data entry. Their disadvantage is, however, that these systems are usually complicated, they are the results of long planning processes, and because of their complexity the probability of failures may be also high. Furthermore, before data recording we have to make sure that the automatic system estimates well the true behaviour, that is, the data collected by the system are in accordance with data collected by an observer.

2.5 Reliability and validity of measurements

Measurements are subject of two kinds of errors: **systematic and random errors** (Fig. 20.3). Systematic error represents the difference between the true value of the variable and its measured value, i.e. **the validity of the measurement**, whereas random error represents errors occurring during measurements, .i.e. **the reliability of the measurement** (Martin and Bateson, 1993). For example, systematic error is, if a thermometer always shows 3 degrees less than the actual temperature because it was miscalibrated (the zero line was drawn at +3 °C). Whereas random error is, if the scale on our thermometer is given only at every 5 °C, therefore our readings are not accurate, and repeated readings do not agree.

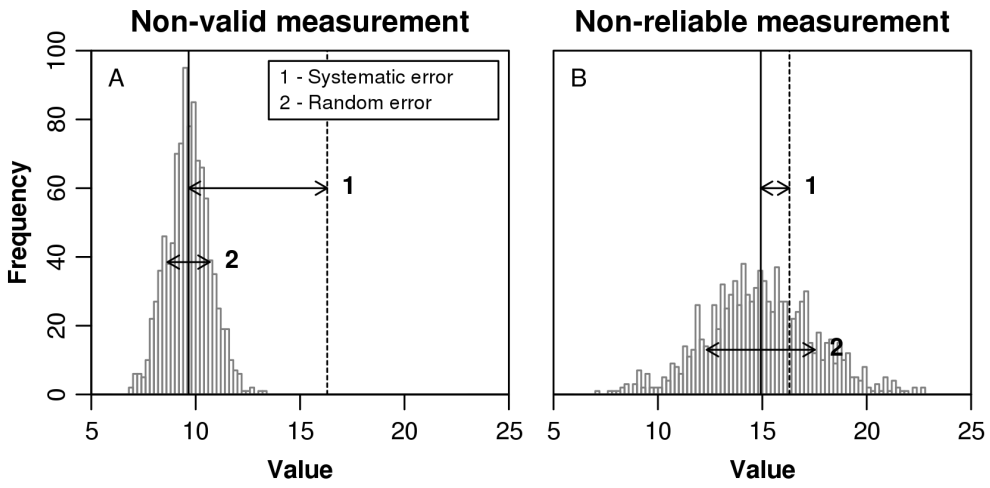


Figure XX.3. Systematic and random errors of measurements contributing to the validity and reliability of estimation. 1000 measurements of a variable with true value of 16.3 (dashed vertical line) with non-valid (inaccurate) measurement (A) and non-reliable measurement (B). In case of non-valid measurement the mean of measured values (solid vertical line) is far from the true value, whereas in case of non-reliable measurement the variance of the measurements is large.

2.6 Agreement between and within observers

The observers can be regarded as instruments that measure a given parameter of the behaviour the same way based on the same principles. To return the thermometer example, as there can be systematic error between two thermometers because one of them is miscalibrated, there can be systematic differences between two observers, because, for example, they interpret and use the definitions consistently differently. Furthermore, as there can be random error in the value read from two thermometers with different scaling, there can be random error between two observers, because, for example, one of them is less experienced or less concentrated, and thus data collected by this observer contain more errors.

Therefore, if our data were collected by several observers, then before data analysis we have to ensure whether the agreement between the sets of data collected by different observers is adequate (**inter-observer agreement or reliability**, Martin and Bateson, 1993). To test this, two observers have to evaluate the same behaviour sequence in real time or from video footage, and we have to compare the resulting data.

The reliability of data collection has to be checked even when data were collected by only one observer. In this case, we examine the degree of agreement of the observer with himself/herself (**intra-observer agreement or reliability**): the observer evaluates the same behaviour sequence twice and we analyse the agreement between the two codings.

If all data were collected by one observer, even then it may be worth to test the inter-observer agreement by including an independent observer. This way it can be detected if the data collected by our single observer has systematic errors similarly to the case when we collect all data with a miscalibrated thermometer.

2.7 Methods to test the agreement between observers

There are several methods to measure the reliability between observers (Martin and Bateson, 1993). Here we review the three most often applied methods.

2.7.1 Correlation between observers

The degree of agreement can be estimated often by **correlation** between the two sets of data. The degree of association between two sets of data is measured by the **correlation coefficient** (r) in which the value can vary between -1 and +1. If $r = +1$, then there is full agreement between the two datasets. With decreasing r, the degree of

agreement decreases, and if $r = 0$, there is no linear association between the two datasets. If $r < 0$, then the two datasets describe the given behaviour in an opposite way.

If the variable follows **normal distribution**, then **Pearson correlation coefficient** (r) is used, otherwise **Spearman rank correlation coefficient** (r_s) can be used in which the value can vary also between -1 and +1.

If we calculate the correlation coefficient by software, then usually the statistical significance (p) is also reported that refers to the divergence of the coefficient from zero. It is important to emphasize that the p value in itself does not give too much information about the degree of agreement, because the significance level of a given correlation coefficient decreases sharply with increasing sample size. Usually we consider the association between the datasets of observers reasonable, if $r \geq 0.7$. If r is lower than this value, then we cannot combine the data collected by the two observers, and either we need to redefine the definitions used for coding or the coding experience of observers has to be improved.

It is important that for the calculation of correlation **independent data pairs** have to be used. That is, it is not correct to calculate the correlation from one sample, i.e. from data gained from sections of one behaviour sequence (e.g. one video footage), but data gained from separate samples have to be used. Furthermore, the sample sections used for the calculation of correlation should be random and representative regarding our sample, otherwise we can easily obtain misleadingly high agreement between two observers, if for example we choose sections where the behaviour in question does not occur at all or occurs continuously.

2.7.2 Index of concordance (KI)

Index of concordance is usually used if the investigated variable was measured on nominal or ordinal scale (Table 1). To calculate the index of concordance we first count the cases where the coding of the two observers agrees (A), and the cases where it disagrees (D), then we calculate the index: $KI = A/(A + D)$ that can have values between 0 and 1.

A shortcoming of the index of concordance is that it does not take into account that some amount of agreement can occur between the observers just by chance, and therefore it may overestimate the agreement between observers.

2.7. 3. Cohen's Kappa (κ)

In contrast to the index of concordance, in the calculation of Cohen's Kappa the number of agreements occurred by chance are taken into account: $\kappa = (KI - V)/(1 - V)$, where KI is the index of concordance, that is, the proportion of agreement between the two observers, and V is the amount of agreement expected by chance.

Let's assume that two observers ('A' and 'B') analysed a 10 minute long footage with sampling every 10 seconds ($n = 60$ sampling points in total). The observers recorded whether a dog on the footage barks or not. From the 60 sampling points in 30 cases both of them found that the dog barked, whereas in 25 cases both of them found that the dog did not bark. Thus the index of concordance is $KI = (30 + 25)/60 = 0.917$.

To determine the value of V, first we need to count how many times from the 60 cases observer 'A' coded barking (A^+) and non-barking (A^-), and similarly we need the values of B^+ and B^- for observer 'B' (Figure XX.4).

		B observer		
		barks (B^+)	does not bark (B^-)	total
A observer	barks (A^+)	30	3	33
	does not bark (A^-)	2	25	27
	total	32	28	60

Figure XX.4. Calculation of Cohen's Kappa in case of a sample consisting of n=60 sampling points.

The probability that two observers code the same assuming independence: $V = A^+/n \times B^+/n + A^-/n \times B^-/n = 33/60 \times 32/60 + 27/60 \times 28/60 = 0.293 + 0.210 = 0.503$. Therefore Cohen's Kappa: $\kappa = (0.917 - 0.503)/(1 - 0.503) =$

0.833. That is much lower than the index of concordance, and shows that almost 10% of the agreement between the observers is due to chance.

Similarly to the correlation coefficient, also in case of Cohen's Kappa there is no objective threshold above which the agreement is considered appropriate. Usually Kappa values above 0.6 may be already considered as acceptable, however, the closer it is to 1, the more reliable the analysis of the behavioural variable is.

2.8 Descriptive statistics

After we checked the reliability of our data, we can start with data analyses in which the first step is the description of the data (Fig. 3). The **localization of the collected sample**, that is, where our sample sits on the axis, is most often characterized with the **mean**. Another often used descriptive statistic of the sample localization is the **median** that is the value at the half of the rank ordered sample.

The **dispersion of our sample**, that is, how wide the data are spread on the axis, is characterized most often with **standard deviation** (s) or with **its square, the variance** ($s^2 = \sum (x_1 - \bar{x})/(n-1)$), where x_i are the individual data points, $\bar{x}$ is the mean of the sample, and n is the sample size. The dispersion of the data can also be characterized by the interquartile range that contains 50% of the data and its calculation is $IQ = Q_3 - Q_1$. Q_3 is **the upper**, whereas Q_1 is **the lower quartile,** the medians of the two sub-samples split by the median of the full data (Fig. 20.5).

Figure XX.5. Localization and dispersion of the sample: the time spent on the nest by the male and the female in an imaginary bird species (n = 10 pairs). On the boxplot the middle line is the median (M), the bottom and top of the box are the lower (Q_1) and upper (Q_3) quartiles, the „whiskers" are the minimum and maximum values in the ranges $Q_1 - 1{,}5 \times (Q_3 - Q_1)$ and $Q_3 + 1{,}5 \times (Q_3 - Q_1)$. Values outside of these ranges are called outliers or extreme values and are depicted with a dot or asterisk.

2.9 Statistical hypothesis testing

The statistical hypothesis is different from the scientific one. The scientific hypothesis is a logical framework that is based on our previous knowledge, and from which predictions can be drawn. In turn the statistical hypothesis is a simple statement pair about a characteristic of the statistical population. The null hypothesis (H_0) states the absence of difference, whereas the alternative hypothesis (H_A or H_1) states the presence of difference. The members of the hypothesis pair should exclude each other, i.e. if H_0 is not true then H_A should be true and *vice versa*.

During statistical testing first we calculate the value of the test statistic from our sample (this is most often done with a statistical software[1]). From this value we can determine the probability (p) to get a value as high (or higher) for test statistic if H_0 is true. If this is highly unlikely, that is, if p is small, then we reject H_0 and accept H_A. If the probability is high then we keep H_0. The probability that is used as the threshold for rejection of H_0 is the **level of**

[1]See Chapter 21 InStat introduction

significance and denoted by α. In biology the widely accepted level of significance is $\alpha = 0.05$, that is, $p \leq \alpha$ values are considered significant.

2.10 Normal distribution, testing normality

It is usually typical for the distribution of variables measured on interval or ratio scale that the values are aggregated near the mean and further from the mean less and less values can be found ("bell shaped curve"). Not all distributions of this kind are normal, but many biological data converges to normal distribution if the sample size is large. According to the **central limit theorem**, if from a non-normally distributed population several random samples are drawn, then the mean of these samples converges to normal distribution. Biological variables are usually the result of several different factors; therefore they converge to normal distribution.

Before conducting parametric statistical tests (see 2.11) we have to ensure that the assumption of normality is fulfilled. The most often used test for checking normality is the **Kolmogorov-Smirnov test** that has, however, a very low power, therefore its application is not recommended. The other often used test for checking normality is the **Shapiro-Wilk test** that has a high power if the data do not contain equal values. However, equal values (ties) often occur in biological data, thus the applicability of this latter test is also limited. Therefore, normality is inspected often graphically by the **quantile-quantile plot (Q-Q plot)**. If the distribution of the sample does not diverge largely from the normal distribution, then the theoretical and sample quantiles give a near straight line (Fig. 20.6.).

2.11 Parametric and non-parametric statistical tests

Statistical tests can be divided in two large groups based on the distribution of the variables to be investigated (Précsényi et al., 2000). **Parametric tests**, as it is in their name, estimate a parameter of the investigated population. They assume that the distribution of the investigated variable (or the error) is normal. The power of parametric tests is high (also small differences can be detected), but they have several assumptions and usually they can be used only on variables measured on ratio or interval scale (see Figure XX.2). In contrary, **non-parametric tests** do not estimate a parameter. They do not require normality, but in case of some of them it is assumed that the distribution has a particular shape (e.g. symmetric). Non-parametric tests have fewer assumptions and can be used also on variables measured on nominal or ordinal scale. They have usually lower power then their parametric counterpart, and many, especially the more complex parametric tests, do not have a non-parametric counterpart.

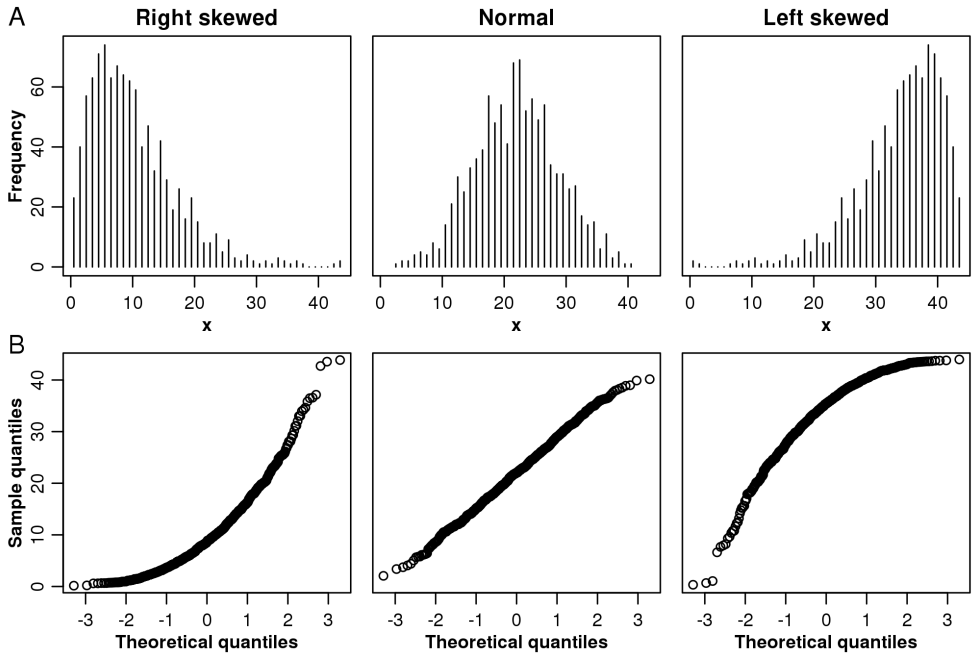


Figure XX.6. Histograms (A) and quantile-quantile plots (B) of right skewed, normal and left skewed distributions.

2. 12 One-sample, two-sample, paired-sample and multiple sample statistical tests

If we have one sample and we intend to compare one of its characteristics to a theoretical value, then we can use **one-sample t-test** (parametric) or **Wilcoxon signed-rank test** (non-parametric). If we have two independent samples, and we intend to compare one of their characteristics, then we can use **two-sample t-test** (parametric) or **Mann-Whitney test** (non-parametric). If we have two samples and the elements of the samples can be arranged into pairs (e.g. before and after treatment values measured from the same individual, males and females of pairs), then we can use **paired t-test** (parametric) or **Wilcoxon paired signed-rank test** (non-parametric). If we have more than two unrelated samples, then we can compare them by **Analysis of Variance** (**ANOVA**, parametric) or by **Kruskal-Wallis test** (non-parametric). If we have more than two measurements from the individuals, then we can apply **repeated-measures ANOVA** (parametric) or **Friedman test** (non-parametric).

2.13 Investigating the association between variables

The linear association between two normally distributed variables can be investigated by **Pearson correlation**, whereas in case of non-normally distributed data we can use **Spearman rank correlation**. Correlation, however, does not assume causality between two variables. If we are interested how an independent variable influences linearly a dependent variable, then we can apply **regression analysis**.

Association between variables measured on nominal scale (e.g. whether the distribution of hair colour depends on sex in humans) can be tested with **test of independence**. In the test of independence usually χ^2 test is used.

2.14 Reporting the results of statistical analysis

When reporting the results of statistical tests, usually we have to give the name of the statistical test used, the value of the test statistic (e.g. t value, χ^2 value), the degrees of freedom of the used sample (parametric tests) or the sample size (non-parametric tests) and the p value.

3. MATERIALS

During the practical we will use video recordings of previous studies carried out by the staff of the department. We will calculate the agreement between observer pairs, and investigate what may influence the degree of agreement between pairs.

4. PROCEDURE

4.1 Task to be carried out during the practical

1. Watching the video footages
2. Defining behavioural variables (one frequency and one occurrence variable)
3. Data recording by two methods (continuous and instantaneous sampling)
4. Describing the data (graph and table)
5. Calculating agreement between observer pairs (Pearson correlation and Cohen's Kappa)
6. Comparing and testing statistically the degree of agreement (e.g. does the distance from the screen influence the results?)

Figure XX.7 Appendix 1. Datasheet for measuring agreement by correlation

REPORT SHEET
Inter-observer agreement: correlation

Date:

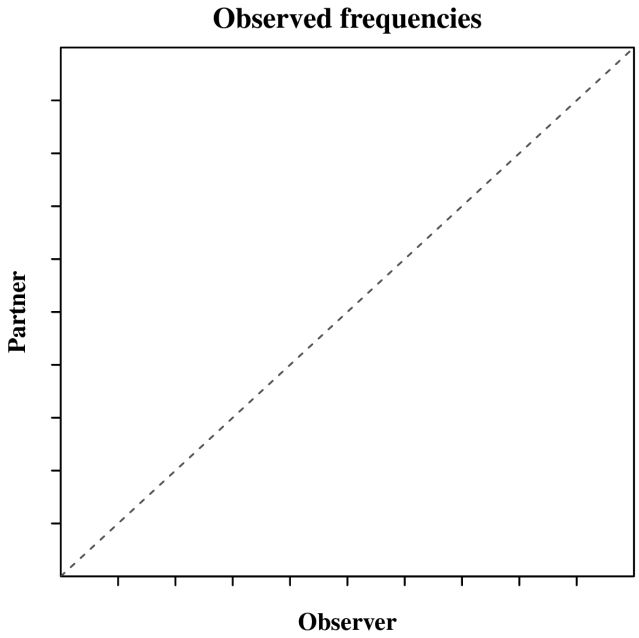
Observer's name: **Partner's name:**

Variable name:

Definition of the variable:

Frequency of the variable in the samples:

Sample	Observer	Partner
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		



Correlation coefficient: r =

Significance: p =

Conclusion:

The relationship between agreement of pairs and the distance to the screen (statistical test and result):

Figure XX.8 Appendix 2. Datasheet for measuring agreement by Cohen's Kappa

REPORT SHEET
Inter-observer agreement: Cohen's Kappa

Date:

Observer's name: **Partner's name:**

Variable name:

Definition of the variable:

Occurrence of the variable:

Time point	Observer	Partner
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		

Time point	Observer	Partner
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
31		
32		
33		
34		
35		
36		
37		
38		
39		
40		

Calculating Cohen's κ:

		Partner		
		$B^+(1)$	$B^-(0)$	total
Observer	$A^+(1)$			
	$A^-(0)$			
	total			

Index of concordance: $KI = \frac{A}{A+D} =$

Agreement expected by chance: $V = \frac{A^+}{n} \times \frac{B^+}{n} + \frac{A^-}{n} \times \frac{B^-}{n} =$

Cohen's κ: $\kappa = \frac{KI-V}{1-V} =$

Conclusion:

The relationship between agreement of pairs and the distance to the screen (statistical test and result):

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Chapter XXI. Practical statistics: how to use the program instat to analyse your data

Vilmos Altbäcker

1. OBJECTIVES

An essential part of most scientific work is to test how your results will predict future events. We will collect data to test our hypothesis regarding the possible causes behind natural phenomena, which is usually some kind of difference, eg. males seem to be larger than females in a certain species, or they tend to be more active, etc. We must compare groups which are similar in most respects but only differ in a certain factor which we believe works as the cause of difference. Then we use statistics to check the probability that our results are obtained by chance alone. If this chance level is low (below 5 percent) then we can conclude that the difference between our groups, and therefore the cause behind the natural variation we want to understand, is probably caused by the factor we test (and not by chance).

During the Ethology Practicals we will generally use the program named InStat by GraphPad Inc USA, which works on any computer running Microsoft Windows 3-7 with at least 3 megabytes of space on the hard drive, and it also runs on Macs. It is pre installed on the PC-s in the computer room of the Ethology Department, but you can also download it from the Ethology dept homepage if necessary. The following chapter is a short compilation of the original Help file of InStat by Graphpad Inc.

2. INTRODUCTION

2.1 Installing GraphPad InStat

GraphPad InStat runs on any computer running Microsoft Windows 3-7 with at least 3 megabytes of space on the hard drive. If you downloaded InStat either from http://www.graphpad.com/apps/index.cfm/demos/download/?demoproducts=IsDemoWin or from the university homepage, you were given installation instructions at that time. Find the file you downloaded using Windows' File Manager or Explorer and double-click on its icon to start the installation process. To remove InStat from your system, run the uninstall program that was installed along with InStat.

To launch the program double click the InStat icon, then the start screen (Fig 21.1.) appears.

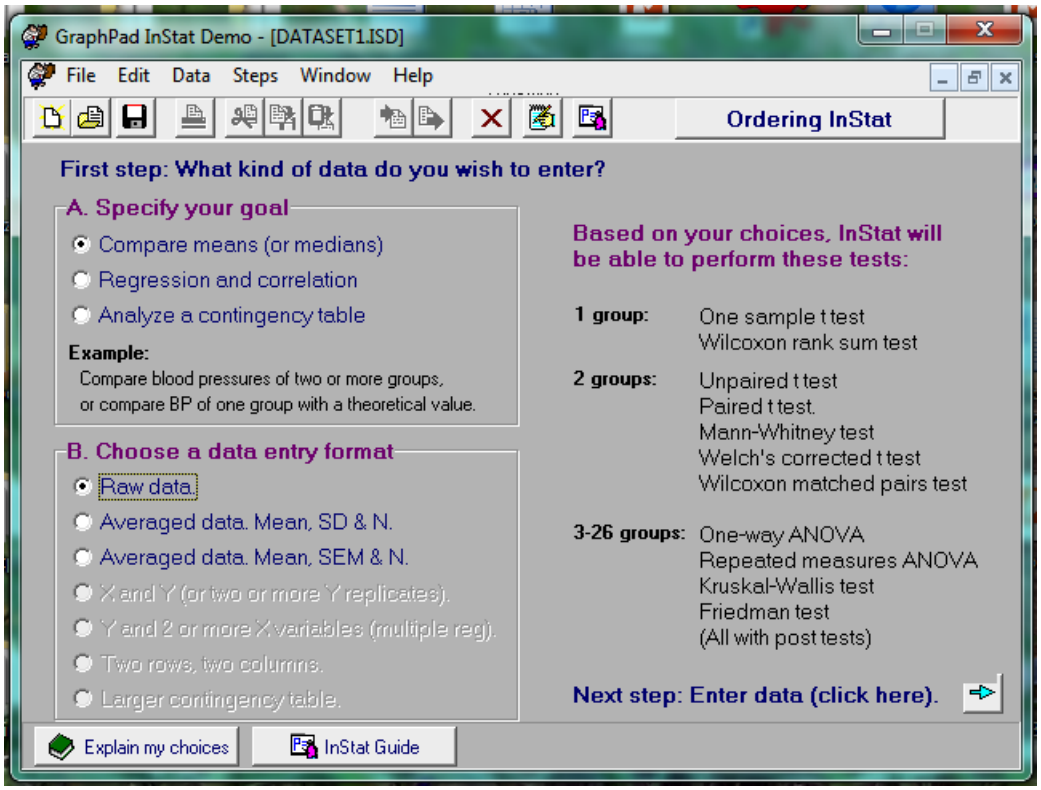

Figure XXI.1. The start screen of the InStat program.

The basic settings of InStat enable you to perform a comparison of two groups with Student t test, which is the most frequently used statistical procedure during the Ethology practices.

2.1.1 InStat toolbar

The toolbar, located directly under the menus, contains shortcut buttons for many menu commands. To find out what a button does, point to the button with the mouse, wait a second, and read the text that appears.

Many menu commands can also be invoked by keyboard shortcuts. When you pull down the menus, InStat shows you the shortcut keys next to the command. The most useful shortcut key is F10, which moves you from step to step.

Click the alternate mouse button (usually the right button) to bring up a menu of shortcuts. You will get different choices depending on which part of InStat you are using.

If you open several documents at once, switch between them by pressing Ctrl- Tab or Ctrl-F6 (or select from the Windows menu).

In order to run the program and perform the test, simply click at the lower right corner.

2.2 The InStat Guide

In order to reduce the learning time, please consult the InStat Guide window, which helps you learn InStat. It appears when you first run InStat, and comes back every time you move from step to step until you uncheck the option box "Keep showing the InStat Guide". Show the Guide again by dropping the Help menu and choosing InStat Guide.

Using the help system

The entire contents of this manual are available in the online help system. InStat uses the standard Windows help engine, so the commands should be familiar to you. Note particularly the button at the right of Help's tool bar

labeled like this: >> Click that button to go to the next help screen. Click it repeatedly to step through every InStat help screen.

2.3. Entering your data

The next screen shows a data sheet, where you need to enter your data either by typing them in or by importing from another program.

Importing data tables from other programs

If you've already entered your data into another program, there is no need to retype. You may import the data into InStat via a text file, or copy and paste the values using the Windows clipboard.

InStat imports text files with adjacent values separated by commas, spaces or tabs. Some programs refer to these files as ASCII files rather than text files. To save a text file from Excel (versions 4 or later) use the File Save As command and set the file type to Text or CSV (one uses tabs, the other commas to separate columns). With other programs, you'll need to find the appropriate command to save a text file. If a file is not a text file, changing the extension to .TXT won't help.

To import data from text (ASCII) files:

1. Go to the data table and position the insertion point. The cell that contains the insertion point will become the upper left corner of the imported data.
2. Choose Import from the File menu.
3. Choose a file.
4. Choose import options.

If you have trouble importing data, inspect the file using the Windows Notepad to make sure it contains only numbers clearly arranged into a table. Also note that it is not possible to import data into a 2x2 contingency table.

Importing indexed data

Some statistics programs save data in an indexed format (sometimes called a stacked format). Each row is for a case, and each column is for a variable. Groups are not defined (as in InStat) by different columns, but rather by a grouping variable.

InStat can import indexed data. On the import dialog, specify one column that contains all the data and another column that contains the group identifier. The group identifiers must be integers (not text), but do not have to start at 1 and do not have to be sequential.

For example, in this sample indexed data file, you may want to import only the data in column 2 and use the values in column 3 to define the two groups.

In the Import dialog, specify that you want to import data only from column 2 and that column 3 contains the group identifier. InStat will automatically rearrange the data.

Filtering data

You do not have to import all rows of data from a file. InStat provides two ways to import only a range of data. You can specify a range of rows to import (i.e. import rows 1-21). Or you can filter data by applying criteria. For example, only import rows where column 3 equals 2, or where column 5 is greater than 100. InStat filters data by comparing the values in one column with a value you enter. It cannot compare values in two columns. For example, it is not possible to import rows where the data in column 3 is larger than the value in column 5.

Exporting data

Transfer data from InStat to other programs either by exporting the data to disk or copying them to the clipboard. Other programs cannot read InStat data (ISD) files.

InStat exports data formatted as plain ASCII text with adjacent values separated by commas or tabs. These files have the extensions *.CSV or *.TXT.

The InStat data table has maximum1000 rows and 26 columns.

Number format

Initially, InStat automatically chooses the number of decimal points to display in each column. To change the number of decimal points displayed, select the column or columns you wish to change. Then pull down the Data menu and choose Number Format and complete the dialog. It is not possible to change the numerical format of selected cells. InStat displays all data in each column with the same number of decimal places.

Altering the numerical format does not change the way InStat stores numbers, so will not affect the results of any analyses. Altering the numerical format does affect the way that InStat copies numbers to the clipboard. When you copy to the clipboard, InStat copies exactly what you see.

2.4. Working with the data table

Editing values

To move the insertion point, point to a cell with the mouse and click, or press an arrow key on the keyboard. Tab moves to the right; shift-Tab moves to the left. Press the Enter (Return) key to move down to the next row.

Row and column titles

Enter column titles on the data table right below the column identifiers (A, B, C…).

InStat labels each row with the row number, but you can create different row labels. When you enter paired or matched data, this lets you identify individual subjects.

Note: InStat copies exactly what you see. Changing the number (decimal) format will alter what is copied to the clipboard.

When you paste data, InStat maintains the arrangement of rows and columns. You can also transpose rows and columns by selecting Transpose Paste from the Edit menu. InStat will paste what was the first row into the first column, what was the second row into the second column and so on.

Deleting data

Pressing the DEL key is not the same as selecting Delete from the Edit menu. After selecting a range of data, press the DEL key to delete the selected range.

InStat does not place deleted data on the clipboard and does not move other numbers on the table to fill the gaps.

Select Delete Cells from the Edit menu to delete a block of data completely, moving other data on the table to fill the gap. If you have selected one or more entire rows or columns, InStat will delete them. Remaining numbers move up or to the left to fill the gap. If you have selected a range of values, InStat presents three choices: Delete entire rows, delete entire columns, or delete just the selected range (moving other numbers up to fill the gap).

To delete an entire data table, pull down the Edit menu and choose Clear All.

Selecting columns to analyze

With InStat, you select columns to analyze on a dialog. Selecting columns on the spreadsheet – as you would to copy to the clipboard – has no effect on the analyses.

Note: Be aware that InStat erases the original data during the transformation. There is no undo command, but you can get back the original data by importing them again.

2.5. Compare groups

InStat will analyze all the columns you entered as the default option. If you want to analyze only particular columns, click the "select other columns" button on top of the screen, where you have chosen the test type. After you read the results, you may want to do the following:

2.6. Print or export the results

Print or export the results (as a text file) using commands on the File menu. Or select a portion of the results, and copy to the Windows clipboard as text.

2.7. View a the results as a graph in InStat

InStat displays a notebook quality graph of your data to help you see differences and spot typographical errors on data entry. You cannot customize the graph to create a publication quality graph. Print the graph or export it as a Windows Metafile (wmf) using commands on the File menu. Or copy to the clipboard, and paste into another program.

2.8. Record notes or append the results to the notes window

Click the Notes button, or pull down the Edit menu and choose View Notes, to pop up a notes editor. Use the notes page to record where the raw data are stored, why you excluded values, what you concluded, or why you chose certain tests. InStat saves the notes as part of the ISD file, so you can refer back to them after opening a file.

To append portions of the results to the notes window, first select a portion of the results. Then pull down the Edit menu and select Append to notes. If you don’t select a portion of the results first, InStat appends all the results.

To print notes, click the alternate (right) mouse button and choose Print.

2.9. Analyze the same data with a different test

Each InStat file contains a single data table and the results of a single statistical test. If you want to analyze data in several ways (say to compare a parametric test with a nonparametric test), you have two choices.

The easiest approach is to simply replace one set of results with another. After completing the first analysis, consider appending the results to the Notes window. Then go back to the Choose test step and make different choices. Then click Results to see the answer. The new results will replace the previous results.

2.10. Perform the same analysis on new data

To perform the same analyses on a new set of data, go back to the data table and replace the data. Then go straight to results. You do not have to select a test, as InStat will remember your choices. The new results will replace the previous results.

An InStat file contains not only a data table, but also your analysis choices. This lets InStat recalculate the results when it opens a file. If you perform the same analysis often, create an analysis template. To do so, simply save a file after deleting the data. The file will contain only analysis choices. To use the template, open the file, enter data and go straight to results. You can skip the Choose Test screen, as InStat reads the choices from the file.

2.11. InStat files and formats

Save an InStat file using the File Save command, and then open it using File Open. The toolbar has shortcut buttons for both commands.

InStat files store your data table along with analysis choices and notes. InStat files are denoted by the extension .ISD (InStat Data). Note that each file contains only one data table.

If you want to share data with other programs, use the File Import and Export commands. Other programs will not be able to open InStat ISD files.

LITERATURE CITED

www.graphpad.com/instat_guide.pdf