FEMALE RESEPTIVITY, SONG REQUIREMENT AND PREFERENCES IN DROSOPHILA VIRILIS AND D. MONTANA

EIJA ISOHERRANEN
Department of Biology

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Academic Dissertation to be presented with the assent of the Faculty of Science, University of Oulu, for public discussion in Raahensali (Auditorium L 10), on March 19th, 1999, at 12 noon.
The truth is inside me
like it is in every living organism
from the fly to the human being.

Feeling deeper pain,
feeling deeper joy,
knowing that every day
I am little closer to my truth.

E.I.

To my daughter,
Isa-Miia
Abstract

Most models of sexual selection focus on coevolution of male sexual trait and female preference for the trait. However, whether the female preference modifies the male trait depends on the overall receptivity of females, on the importance of the male trait for the females, on female sampling behaviour, and on female control over copulation decision. These aspects are often neglected by theoreticians.

Female receptivity, song requirement and preferences were studied in two Drosophila virilis group species, D. montana and D. virilis. The main object of the study was female acceptance/rejection behaviour. Female wing spreading posture was a signal for males to attempt copulation, when the female was ready to mate. I used this signal as an indicator of female acceptance. D. virilis females were generally very receptive, but there were differences between females both in receptivity and in responsiveness of the females to simulated courtship songs. D. virilis female did not require song and had a low acceptance threshold with a heterospecific male. These two traits are explained by a high female receptivity. D. montana females, on the contrary, had a high acceptance threshold. These females accepted the courting male only after hearing his song. They also repelled males, which attempted copulation without female acceptance signal. In this species the strength of species discrimination did not correlate with the overall receptivity of the females. Between species hybrid females (from a cross vir x mo and from backcross to mo) resembled D. montana females in their song requirement, but not in their receptivity. This suggests that these two traits are inherited independently.

D. montana and D. littoralis females have previously been found to prefer males with short and dense sound pulses in wild. These song characters were repeatable among overwintered males in a fashion different from other song characters. This shows that song characters involved in sexual selection are more sensitive to environmental factors than other song traits.

Keywords: courtship, acceptance signal, species recognition, inheritance
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Oulu, February 1999

Eija Isoherranen
**Abbreviations**

FRE = the carrier frequency of the song, determined from Fourier spectries
CN  = the number of cycles in a sound pulse
IPI  = the interpulse interval, i.e. the time from the beginning of one sound pulse to the beginning of the next one
PL  = the length of a sound pulse
PN  = the number of pulses in a pulse train
PTL  = the length of a pulse train
List of original publications

This thesis is based on the following publications, which are referred to in the text by their Roman numerals.


IV  Isoherranen E, Aspi J & Hoikkala A. Inheritance of species differences in female receptivity and song requirement between Drosophila virilis and D. montana. Submitted in Anim Behav.
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1. Introduction

Most sexually reproducing species possess a species-specific, eventful and much effort and time consuming repertoire of signals exchanged between the courting pair. Yet many courtships do not lead to copulation. Complex courtship rituals make it possible for the female to exercise species discrimination and to choose the best available conspecific male as her mating partner.

1.1. Theories of sexual selection

Coevolution of conspicuous male traits and female preferences on these traits has invoked a lot of interest among theoreticians. Stages in communication between a sender and a receiver can be divided into two parts, signal design and signal function. Natural selection acts mainly on signal design (e.g. communication efficiency) and sexual selection on signal function (signal content, Endler & Basolo 1998).

One of the questions in the theories of sexual selection is why the females use so much energy and time to choose their mate, i.e. do the females gain direct or indirect benefits from their choice. When the male takes part in parental care and/or gives the female other resources, choosing a good mating partner increases the female’s own viability and fecundity (direct benefits).

There are two major sexual selection theories that are based on function of male courtship signal in female mate choice. Females are predicted to gain benefits only through offspring survival and fecundity. Both theories assume that there is heritable variation in the preferred male trait and in the female preference. The “arbitrary traits” model of sexual selection (e.g. Fisher 1958, Pomiankowski et al. 1991) assumes that the preferred male trait does not give the female any information on male quality. According to this model, by mating with a male with a preferred character, the females can increase the mating success of their sons among the females with the same kind of preference. In the “viability indicator” model (good genes), in particular in its “conditional” variant (Zahavi 1977, Iwasa et al. 1991), sexually selected traits are assumed to help the females to choose a good-quality male on the basis of the male sexual traits. The preference for the male trait is selected for, because the offspring of
the females exercising selection tend to be more viable than that of the rest of the females.

In the “sensory exploitation” model (Ryan & Rand 1990), biases in female preferences (for an already existing trait) evolve without female sexual selection on the trait. For example, females could benefit by using the same signal-perception system in other contexts in detection of food (Endler & Basolo 1998). Later, the female preferences for new trait variants may lead to a modification in male trait. Consequently, female preference and male sexual trait do not evolve in a correlated fashion.

Sexual selection theories are based on female preferences on male signal trait. The mating propensity of males and females in species having complicated courtship rituals depends, however, on factors working at three levels: (1) sexual drive of the courtship partners, (2) female (and male) requirement and/or predilection for specific signals and (3) female preference for specific characters of the male signal. Two first levels are often neglected by theoreticians.

1.2. The sexual drive of flies

The sexual vigour of males and receptivity of females are influenced by the internal factors of individuals themselves as well as by the quality of the courtship partner and the signals emitted by him/her. When environmental factors, including the quality of the mating partner and the emitted signals, are randomised or standardised, one can estimate the genetic factors of sexual drive. In *Drosophila*, male sexual vigour and female receptivity can be studied by observing whether and when the flies indicate their readiness to mate e.g. by observing the copulation attempts of males and the acceptance signals of females. When detailed information on female receptivity is needed, one can measure the duration or intensity of courtship, the amount of courtship signals produced or the energetic value of resources the male gives to the female.

In insects, like in almost any sexually reproducing animals, the sexual drive of females is lower than that of males (Thornton & Alcock 1983). Why does a female sometimes consistently refuse to mate with a courting male? Females invest in the offspring a lot by producing a few large and nutritious eggs in comparison to males, which produce huge numbers of small sperm. Because mating is costly for the females of many species also in terms of future survival (e.g. Turner & Anderson 1983, Partridge & Fowler 1990), it is beneficial for the females to invest in offspring quality and/or in resources the male is offering to them (Trivers 1972, Clutton-Brock 1991). Females with a high acceptance threshold gain benefits for herself or for her progeny by exercising choice between their prospective mating partners. Holland and Rice (1998) offer another interesting explanation for the low receptivity of females by suggesting that females evolve resistance to male display traits, because attractive males induce females to mate in a suboptimal manner. The lower sexual drive of females in comparison to that of males gives the males two evolutionary ways to achieve more matings: to evolve traits enhancing forced copulation (Thornhill 1980, Rowe et al. 1994) or to evolve traits that raise female receptivity during courtship.

Whether the female has a control on copulation decision depends on the effectiveness
of her refusal and acceptance signals. The female may indicate her unwillingness or readiness to mate at several courtship stages. *Drosophila* female can influence the behaviour of the courting male by standing, walking, preening, etc. (Welbergen et al. 1987). She can also indicate her unwillingness to mate by emitting specific repelling signals: decamping, kicking, wing fluttering, abdomen elevation and/or depression, and extrusion (extending and elongating the tip of abdomen, Spieth 1974). If the male attempts copulation before the female is ready to mate, she may try to dislodge male and break off copulation before any sperm has been transferred. In many *Drosophila* species, females have a special acceptance signal, which they have to give before the male attempts to copulate with them (Spieth 1974). Females may indicate their readiness to mate by spreading their wings, by spreading their vaginal plates and/or by extruding their ovipositor (Spieth & Ringo 1983). Despite of the large signal repertoire in courtship, *Drosophila* female receptivity has been studied by observing female signals, and not copulation itself, in only a few studies (Ikeda et al. 1981, Tomaru et al. 1995, Ritchie et al. 1998).

One important current issue in the study of sexual isolation is the connection between species recognition and the behaviour of the flies in within species courtship. There is much support (Faugeres et al. 1971, Carracedo & Casares 1987, Pineiro et al. 1993, Izquierdo et al. 1992) to the idea that, in *Drosophila*, female receptivity with conspecific males may have played a major role in the evolution of factors maintaining sexual isolation between species. Carracedo et al. (1991) have also suggested that the sexual isolation between a *D. melanogaster* female and a *D. simulans* male and the receptivity of a *D. melanogaster* female in between species courtship are determined, at least in part, by the identical genes or by closely linked gene clusters. The hypothesis that species recognition system and female receptivity are parts of the same process requires further testing with species differing from each other in the two traits. Females of two closely related *D. virilis* group species, *D. virilis* and *D. montana*, are ideal objects for testing this. *D. virilis* females are very receptive in within and even in between species courtship, while *D. montana* females require a long courtship and mate only with conspecific males (Hoikkala 1988, Liimatainen & Hoikkala 1998).

### 1.3. Female requirement and predilection for specific signals

To understand mate choice fully, we must be aware of the full set of cues used in this choice and their relative importance of these cues in sexual arousement (Widemo & Sæther 1999). The male eagerness to court and the female readiness to accept the courtship are influenced by the requirements of the flies of both sexes for species-specific signals. In *Drosophila* species, the courtship consists of a complicated signal-response chain between the male and the female, including four kinds of stimuli: acoustic, olfactory, visual and tactile (Ewing 1983, Spieth & Ringo 1983). In many species the courtship relies mainly on one or two signals, and the lack of these signals may block the whole courtship and prevent copulation (Ewing 1983).

The role and importance of specific signals may vary from species to species, and the ultimate importance of presence or absence of the signal may be defined on two levels.
In my thesis signal requirement is defined as the signal being a prerequisite for the females' readiness to accept the courtship or the males' readiness to attempt copulation. I also predict that the requirement is fixed in populations or species. Some courtship signals may not be a requirement for copulation, even though they stimulate the flies to mate. Consequently, I have defined the female to have a predilection for a signal, if the reception of this signal reduces the amount of courtship the female requires before she accepts the courting male. Signal requirement and predilection are measured on the level of presence or absence of the signal. These two traits can best be studied in laboratory by manipulating males (blocking the signal channel or adding a new signal in the signal repertoire).

The evolution of a specific signal has been usually assumed to require coordination between the signal and the receiver (Alexander 1962, Butlin & Ritchie 1989). Pre-existing bias model (Basolo 1990), however, emphasises the evolution of novel traits as a result of inherent biases in the sensory system of female. In some species the females have been found to have a predilection for a signal, which is absent in conspecific males in wild (Basolo 1990, Morris & Ryan 1996, Ryan et al. 1990). In general, signalling systems of animals are strongly connected to other aspects of species ecology through evolution of the sensory system and brain (Endler & Basolo 1998).

Courting Drosophila males produce auditory signals by vibrating their wings. These signals have been found to affect female responsiveness and mate choice in several Drosophila species (Ikeda et al. 1981, Crossley & Bennet-Clark 1993, Aspi & Hoikkala 1995, Ritchie & Gleason 1995), which suggests that the females have a predilection for courtship song. D. montana, D. ezoana (Hoikkala 1988, Liimatainen et al. 1992) and D. busckii (Bixler et al. 1992) females reject wingless (mute) males regardless of how intensively they are courted. This means that the females of these species have also a requirement for male auditory signals.

The strength of sexual isolation between closely related species may vary depending on whether the females require species-specific courtship signals. Sympatric species are often adapted to use different signals or signal combinations in their courtship (Hoikkala et al. 1994). Species-specific auditory signals serve as isolation mechanism in many species of frogs and toads (e.g. Blair 1974, Marques & Bosch 1997), birds (e.g. Neuchterlein 1981) and insects (e.g. Lee 1983, Crossley 1986). In northern populations of four D. virilis group species, D. lummei, D. ezoana, D. montana and D. littoralis, between species courtships are common (12 % of all observed courtships) in wild (Aspi et al. 1993). These courtships never lead to copulation, contrary to the courtships in laboratory in a no-choice situation. Heterospecific courtships in wild break off, when the male begins to sing. This suggests that hearing a heterospecific courtship song prevents females from accepting the courting male (Liimatainen & Hoikkala 1998).

In studies tracing the evolution of factors leading to sexual isolation between species, genetic studies on female requirement and predilection for courtship signals are of paramount importance. What kind of genetic changes accompany the divergence of species-specific recognition system? Speciation could result from an accumulation of small changes at many loci (Charlesworth et al. 1982) or from a major genetic reorganisation (Carson & Templeton 1984). One of my goals has been to find out what is the relationship in female receptivity between intra- and interspecific courtship and what is the role of female requirement and predilection for male courtship song in this
context. *D. virilis* and *D. montana* are again ideal material for studying this question: *D. virilis* females occasionally accept a wingless (mute) male after a prolonged courtship, while *D. montana* females never mate, if they do not hear the song produced by the courting male (Hoikkala 1988, Liimatainen et al. 1992).

### 1.4. Female preference for specific characters of a male signal

Heisler *et al.* (1987) defined the female mate choice as differential mating by females that results from mating preferences. Andersson (1994) has reviewed multitudes of studies showing that females prefer some male trait variants over others and that males with preferred traits have an enhanced mating success. While female signal requirement and predilection are defined at the level of the presence or absence of a certain signal, female preference is defined at the level of the variation of the male signal trait that is a target of female choice.

Wagner (1998) has proposed that female mate choice is differential mating that results from the interaction of mating preferences, environmental conditions, and sampling strategies. There must be a balance between the benefits of finding the best male from a group of males and a disadvantage of wasting time and energy in sampling potential mates. For instance, *D. montana* and *D. littoralis* females prefer males with short and dense sound pulses (high carrier frequency) in their courtship song (Aspi & Hoikkala 1995, Ritchie *et al.* 1998). Nevertheless, *D. montana*, *D. ezoana* and *D. littoralis* females accept a lower quality male (with respect to male courtship song) as their mating partner, given that there are no better males available (Hoikkala & Aspi 1995).

The female preferences are amply documented on the population level (Møller & Zamora-Munoz 1997, Sheridan & Pomiankowski 1997, Tobias & Hill 1998, Ritchie *et al.* 1998). Given, however, that we ask questions such as (1) what kind of preference do individual females have (narrowly or broadly tuned), (2) are there between female differences in their preferences and (3) has there been coevolution between the female preferences and the preferred male character, this approach is quite inadequate. To study these questions in more detail, we need a specific measure to describe female preference (Wagner 1998). “Window of preference” is the rate at which the probability of a response to signal declines when the signal deviates from individual female’s mean preference (Butlin 1993). Preference function also describes the shape of female preference. It is a common feature in genetic models of sexual selection (e.g. Lande 1981, Kirkpatrick 1982, Heisler 1984b), but quite rare in empirical studies measuring female preference (but see Basolo 1995, Wagner *et al.* 1995).
1.5. Aim of the present research

My aim was to study the quality of the song of the courting males and the responses of the courted females. I strive to pay special attention to the receptivity, song requirement and preferences of the females. The studies have been done on D. virilis group species, mainly D. montana and D. virilis. In paper I we have studied whether the female wing spreading signal can be regarded as an acceptance signal. We also have tried to find out whether the males need additional stimuli from female genitalia to attempt copulation. Finding a signal indicating females’ readiness to mate was a prerequisite for studies on female song preferences (paper III).

D. montana and D. littoralis females have earlier been found to exercise choice on the basis of the pulse characters of the male courtship song in wild (Aspi & Hoikkala 1995). In paper II we have studied variation and repeatability of courtship song characters among wild-caught and laboratory-reared males. We wanted to find out how sensitive different song traits are to environmental factors, and how much the songs of the males may change before the mating season of the flies in wild.

The aim of the papers III and IV was to focus on female requirements in courtship. In paper III we played simulated courtship songs to D. virilis females and observed their responses (wing spreading) to these songs in order to study variation and consistency of female preferences on male song traits. In the last paper (IV) our aim was to study the genetic basis of species differences in female receptivity and female song requirement. Here we made crosses between D. virilis and D. montana differing from each other in the two above mentioned characters.
2. Materials and methods

The materials and methods have been described in detail in the original papers (I, II, III, IV); only a brief outline is provided here.

2.1. Flies

All flies used in papers I, III and IV were collected from laboratory strains of *D. virilis* group species. In study II we also used wild-caught *D. montana* and *D. littoralis* flies and their F₁ progeny. They represented the wild populations of these species.

The fly strains were maintained in culture bottles containing Lakovaara’s (1969) malt medium in continuous light, at 19°C. Freshly emerged flies were sexed under light CO₂ anaesthesia and the males and the females were collected in separate food vials. At the age of two to three weeks males and females were considered to be sexually mature and were used in experiments.

In experiments reported in papers I and IV we made use of manipulated flies (wingless flies and/or females with blocked genitalia). Manipulations were made under a light CO₂ anaesthesia one day before the flies were used in experiments.

In paper IV we made hybrids between *D. virilis* and *D. montana* to study the inheritance of female behavioural traits. Species crosses were conducted by placing about two weeks old virgin males and females of different species in the same culture bottles.

2.2. Observation and recording techniques

In paper I, we observed the mating behaviour of flies of both sexes in a no-choice situation using a dissecting microscope. We measured the length of active courtship time until copulation, and registered licking behaviour and copulation attempts of the males and wing spreading of the female.

To study the repeatability of the song characters of wild-caught (fresh and overwintered) and laboratory reared *D. montana* and *D. littoralis* males, we recorded the
song of each male on two different days (paper II). For the laboratory reared males the song recordings were made twice also after keeping the males in a cold room at 4 Cº. Recordings were made with a JVC condenser microphone and a Sony TC-FX 33 cassette recorder. The songs were analysed with a Gold 1425 digital oscilloscope and with a Signal Sound Analysis System (Engineering design). The song of D. montana consists of trains of sound pulses. D. littoralis males produce also sound pulses in trains, but the lengths of the pulse trains and the intervals between sound pulses are long and irregular. We analysed the pulse characters, e.g. calculated the number of cycles in a pulse (CN), and measured the lengths of the sound pulses (PL) from oscillogram and analysed the carrier frequency of the song (FRE) from Fourier spectra, using the Signal sound Analysis System (Engineering design). For D. montana, we also measured the lengths of the pulse trains (PTL), the number of the pulses in a train (PN) and the distance from the beginning of one pulse to the beginning of the next pulse (interpulse interval; IPI).

In paper III we used another technique to measure female preferences for different song characters. We studied the responses of D. virilis females to simulated courtship songs by observing whether and when the females show their acceptance by spreading their wings in song simulation experiments. These studies were made without any males being present. The simulator used to produce songs was based on a programmable sound generator connected to the I/O space of a personal computer (Aspi et al. 1992). We played species-specific song and two kinds of modified songs for each female: one, where the IPIs were about 10 standard deviations longer than those in the song of the strain used in experiments, and one, where both IPIs and PLs were 10 standard deviations longer than the ones in the song of the above mentioned strain. The responsiveness of individual females were observed on four successive days (modified songs once and species-specific song twice). In this study the females were introduced into a special chamber prepared from a Petri dish. The chamber was placed on top of a loudspeaker through which the simulated courtship songs were played.

To observe female courtship behaviour in paper IV, female and male were aspirated in to a circular plastic cell. We measured female receptivity by recording for each courtship the durations of all courtship bouts of the male before the female spread her wings and by paying attention also to female locomotion. Female acceptance speed refers to the sum of the durations of male courtship bouts (active courtship) prior to female wing spreading. The receptivity of individual female was observed on three or four successive days with males of different geno- or fenotypes. As soon as the male achieved intromission, the flies were gently separated, because the messages transferred along with sperm could greatly affect the female receptivity (Ringo 1996).

DeBenedictis (1977) and Koepfer (1987) have defined fly mating propensity as the probability that an individual will mate within a given period. Our female mating propensity (paper IV) differs from this definition in that we observed female acceptance signal (wing spreading) instead of copulation, and that we took in account only the trials, where the male began to court the female. Consequently, our female mating propensity and acceptance speed was not affected by variation in male sexual vigor and by forced copulations. We also observed female receptivity in more detail by recording for each courtship the durations of all courtship bouts of the male and by paying attention also to female locomotion.
2.3. Statistics

Repeatability analysis was used to study consistency of male courtship characters (paper II) and female wing spreading response for simulated songs (paper III). The repeatability assay identifies the degree to which variation among individuals explains total variation in the studied trait (Falconer 1989). It sets an upper limit for the heritability of a trait (Boake 1989), and it also shows the potential utility of the signal as a reliable indicator of competitive ability of the males. In addition to repeatability, we calculated Goodman and Kruskal’s predictive ability for the female wing spreading data (Everitt 1977).

Female responses to simulated songs were analysed by subjecting the female response data to a logit analysis. The GLIM statistical package (Aitkin et al. 1990) was used to fit and determine the parameters of the logit models. We also estimated Akaike’s information criterion for each logit model to select a model with a highest information content (Christensen 1990).
3. Results and discussion

3.1. Female wing spreading as an acceptance signal

To study female receptivity, signal requirement and preferences, we need to know when the female is ready to mate and how she expresses her readiness for the male. This question was studied in paper I by observing the behaviour of the females of 12 *D. virilis* group species or subspecies in a single-pair courtship. Nearly all females spread apart their wings prior to copulation. 80-100% of the males (depending on the species) responded to female wing spreading by a copulation attempt. Female wing spreading clearly helped the males to time their copulation attempts when the female was ready to mate.

The female acceptance posture called wing spreading is a widely distributed phenomenon in the genus *Drosopila* (Sturtevant 1921, Spieth 1952, 1974, Grossfield 1966, 1968). Some well-known species like *D. melanogaster* (Spieth 1974) do, however, not have it. In cases, where the females do not spread their wings actively before copulation, the male pushes female’s wings apart when mounting the female. In paper I we found some *D. virilis* group males to try to copulate with a female, which had not spread her wings (0-33% of all copulation attempts, depending on the species). About half of these attempts were successful. In paper IV, some wingless (mute) F₁ males (from the cross *D. virilis x D. montana*) succeeded achieving intromission without a preceding female wing spreading act. These findings suggest that the males do not require that the female spreads her wings before copulation.

In normal courtship female wing spreading may have a different signal value in different species. Spieth (1952) has suggested that female wing spreading is a facilitating act without any signal function. It has also been suggested that female wing spreading is an effective signal only when combined with some other signals (Ewing 1983), and that female wing spreading suffices to evoke male copulation attempt *per se* (Liimatainen 1993). In paper I, *D. virilis* and *D. novamexicana* males courting a wingless female (and consequently could not see female wing spreading signal) made several unsuccessful copulation attempts and had difficulties in timing their attempts to coincide when the female was ready to mate. In paper IV, *D. montana* and also
backcross (from cross \((\text{vir} \times \text{mo}) \times \text{mo}\)) females, which had not spread their wings prior to male mounting, tried vigorously to get rid of the mounting male. These findings confirm that female wing spreading is a signal and not only a facilitating act.

Females can indicate their readiness to mate also by spreading their genitalia. According to Spieth (1952), this is a primitive acceptance signal, which the males can detect by thrusting their proboscis between the genital plates of the female (‘licking’). In paper I, \(D.\) \textit{americana}, \(D.\) \textit{texana}, \(D.\) \textit{novamexicana} and \(D.\) \textit{lummei} (i.e. the species of \(D.\) \textit{virilis} phylad, except for \(D.\) \textit{virilis}, Throckmorton 1982) males licked female genitalia before and also after the female had spread her wings, before they attempted to copulate with the female. Some males of the \(D.\) \textit{montana} phylad (Throckmorton 1982), however, copulated without licking female genitalia at any courtship stage. As an extreme case, only half of the \(D.\) \textit{borealis} males licked female genitalia prior to copulation attempt. Most of the males that did not respond to female wing spreading signal had not licked female genitalia prior to receiving this signal. This was true for all species studied here.

According to Spieth (1974), licking is an essential component of \textit{Drosophila} courtship behaviour. In paper I, we blocked the genitalia of \(D.\) \textit{virilis} and \(D.\) \textit{novamexicana} females to prevent males from receiving stimuli from female genitalia. This did not prevent male copulation attempts, but it reduced the male’s tendency to respond in female wing spreading. This suggests that in the \(D.\) \textit{virilis} group the male proboscis-female genitalia contact plays a part, but is not always a prerequisite of male copulating attempts (males have a predilection but not a requirement for stimulus from female genitalia). In contrast to males, \(D.\) \textit{virilis} and \(D.\) \textit{novamexicana} females did not seem to have any predilection for stimuli received through male licking. Females with blocked genitalia spread their wings repeatedly even though the males were not able to insert their proboscis in female genitalia. Blocking the genitalia did not affect the length of courtship required by the female (Mann-Whitney U: \(D.\) \textit{virilis}: \(U=33.00, N_1=10, N_2=8, p=0.53\); \(D.\) \textit{novamexicana}: \(U=60, N_1=14, N_2=10, p=0.58\)).

In \(D.\) \textit{melanogaster}, selection for males licking the female at a low rate has been very successful, in contrast to selection for high licking rate males (Welbergen & Dijken 1992). The authors suggest this to be due to past directional selection pressure caused by female preference for males licking at high rate. Welbergen and Dijken (1992) did not find selection for high or low licking rate to have any discernible effect on female mating propensity. Stimuli received from female genitalia may be more important for \(D.\) \textit{melanogaster} males than for \(D.\) \textit{virilis} group males, as the females of the \(D.\) \textit{melanogaster} spread only their genitalia, but not their wings.

Our results (paper I) suggest that in \(D.\) \textit{virilis} group female wing spreading is not an absolute necessity \textit{per se} for a male copulation attempt. It can, however, be regarded as an acceptance signal, as it helps the male to time the copulation attempt to coincide when the female is ready to copulate. In some species of the group this signal seems to be most efficient when combined with stimuli from female genitalia.
3.2. The inheritance of the species difference in female receptivity between *D. virilis* and *D. montana* in courtship with conspecific males

Variation in female receptivity and/or the genetic basis of this variation has been investigated in a few studies only (Ikeda *et al.* 1980, 1981, Welbergen *et al.* 1992, Carracedo *et al.* 1991, Casares *et al.* 1992, Pineiro *et al.* 1993), largely due to difficulties in quantifying female behaviour. We studied the genetic basis of species difference in female receptivity in paper IV by observing the behaviour of *D. virilis* and *D. montana* females and the females of *F*₁ (from cross *vir* x *mo*) and backcross (*BC*₃₉₀; from cross *F*₁ x *mo*) generation with conspecific males (using *F*₁ males for *F*₁ females and *D. montana* males for *BC*₃₉₀ females). 82.4% of *D. virilis*, 55.6% of *D. montana*, 95.4% of *F*₁ and 37.0% of *BC*₃₉₀ females spread their wings when courted by a conspecific male. Differences between the parental species in female acceptance speed and locomotion behaviour were clear-cut. *D. virilis* females required a short courtship (median of acceptance speed 23 sec), during which they were nearly motionless, while *D. montana* females required a long courtship (median 99 sec), and settled down only after a log running period (median 64 sec). *F*₁ hybrid females were intermediate to their parent species in receptivity (acceptance speed and running behaviour). This suggests codominance or polygenic inheritance of this trait.

The proportion of *BC*₃₉₀ females, which spread their wings during the observation period, was quite low (37 %; paper IV). Females, which spread their wings, resembled in their acceptance speed and running and standing behaviour *F*₁ females. Those *BC*₃₉₀ females, which spread their wings when courted by *D. montana* males, did this also when courted by *F*₁ hybrid males. There was also correlation in receptivity (acceptance speed and running time) of the responding females in the two above-mentioned courtships. The extensive variation in the receptivity of *BC*₃₉₀ females is most probably due to a segregation of genetic factors in these hybrids.

Female receptivity to conspecific males is a species-specific feature adapted to environmental factors, like the sexual vigour and availability of the males and the ecological niche of the species. Both *D. montana* and *D. virilis* females have been found to mate repeatedly in laboratory (Hoikkala, personal communication). In *D. montana* the same phenomenon has been observed also in wild (Aspi & Lankinen 1992). Females of neither species seem to get any kind of recourses along sperm from male (Aspi 1992, Pitnick *et al.* 1997), like some *Drosophila* females are reported to get (Markow & Ankney 1984, Steele 1986). Flies of the two species differ, however, in population structure and mating strategy. *D. virilis* is subtrobical and a commensal of culture. The spread of culture has opened up new habitats for them. This process may have included bottlenecks and favoured females with low receptivity. *D. virilis* flies are sexually active throughout their adult life. *D. montana*, on the contrary, is adapted to live in nothern climates. *D. montana* adults have a short mating season after overwintering (Aspi & Lankinen 1992), which suggests that the females invest much per mating in comparison with *D. virilis*.

The chase away model is another possible explanation for the low receptivity of *D. montana* females (Holland & Rice 1998). The model suggests that males with an exaggerated display trait persuade females to mate at a rate beyond the female optimum, which selects for discriminating females with a high stimulatory threshold. According
this model, intense sexual selection of *D. montana* females for male song in the wild (Aspi & Hoikkala 1995) might also have led to high acceptance threshold of these females.

In *D. melanogaster*, there seems to be a strong relationship between the general activity and mating speed of the females (Manning 1961, 1968, Burnet & Connolly 1974). Decamping is a *D. melanogaster* female repelling signal, which usually blocks the courtship (Welbergen 1987). The tendency of the females to slow down towards the end of courtship facilitates copulation (Hall *et al.* 1980, Tompkins *et al.* 1982, Markow 1987). This suggests that female locomotion is a useful predictor of female receptivity also in *D. melanogaster*.

The *D. virilis* courtship may include a running period before the female settles down (Liimatainen 1993). In our study (paper IV) females do not move around during the courtship. Lack of female locomotion in our *D. virilis* strain (eye pigment mutant peach strain) may be a consequence of reduced visual capacity. Males of this strain seemed to be unable to follow the female during the courtship. Tompkins *et al.* (1982) have found the same phenomenon in *D. melanogaster*. When visual cues were removed, the males had difficulties in locating the females. This again affected the locomotion and stopping behaviour of the females. Welbergen *et al.* (1992) have also suggested that the length of courtship is likely to be affected by anomalies in the dynamics of courtship interactions between male and female. Within species variation in fly mating propensity and mating speed has previously been studied only in species, where the females do not have a clear acceptance signal, like *D. melanogaster* and *D. pseudoobscura* (reviewed by Collins & Hewitt 1984).

Carracedo *et al.* (1991) have shown that there are genetic differences in female receptivity between isofemale lines of *D. melanogaster*. Selection for high and low female receptivity and two diallel crosses revealed large additive variation in female receptivity (Pineiro *et al.* 1993, Casares *et al.* 1992). Casares *et al.* (1992) argue that evidently natural selection does not favour females with extreme values of receptivity.

### 3.3. Species discrimination

The mating propensity of *D. montana* and *D. virilis* females was at the same level in the courtships with conspecific and with F<sub>1</sub> (*vir* x *mo*) males. This indicates that the signals of both male genotypes were within the range of acceptable cues (paper IV). The females were, however, less receptive in courtship with F<sub>1</sub> than in courtship with conspecific males. *D. virilis* females required nearly twice and *D. montana* females more than five times longer courtships when courted by F<sub>1</sub> males than when courted by conspecific males. *D. montana* females also ran six time longer time in courtships with hybrid than with conspecific males. These findings suggest that the females discriminated against F<sub>1</sub> males. *D. virilis* females discriminated evidently also against *D. montana* males, even though some females accepted these males.

The low receptivity of *D. virilis* and *D. montana* females in courtships with F<sub>1</sub> hybrid males could be due to female predilection for species-specific signals (e.g. pheromones) or to preference for species-specific or even strain-specific character of signals. In study
IV, the song of inbred males had some strain-specific characters and the song of hybrid males actually resembled the song of wild-caught *D. montana* males more than that of the males of the inbred *D. montana* strain did. Female receptivity could also be affected by the order of signal repertoire. Welbergen *et al.* (1987) and Liimatainen *et al.* (1992) have argued that courtship signals have to be presented at a particular courtship stage, as a response to the behaviour of the opposite sex. Liimatainen *et al.* (1992) have shown that *D. montana* females respond to male touching by standing, which is followed by male licking and wing vibration. In our study (paper IV), *F₁* males had a high sexual vigour and they often began to sing before *D. montana* females had the time to settle down. Robertson (1982) has suggested that short male latency (i.e. time from the beginning of experiment to the first courtship act of the male) leads to long courtship duration, because the female may be too agitated to receive male signals immediately after entering the mating chamber. The *F₁* males had shorter latencies than *D. montana* males (paired samples test: \( t_{17} = -2.289, p=0.035 \)). There was, however, no significant correlation between these males in courtship latency and acceptance speed (Spearman’s correlations: *F₁* males: \( r_s = -0.416, p=0.086, N=18 \), *D. montana*: \( r_s = -0.389, p=0.123, N=17 \)).

Students of the inheritance of female receptivity in courtship with the males of different genotypes in general and *Drosophila* students in particular have seldom cared to pay attention to female signals. For instance, Dow (1976) and Heisler (1984a) have studied the inheritance of mating propensity of *D. melanogaster* females in courtship with the males of *yellow* locus genotypes without paying any attention to female locomotion or to her repelling or acceptance signals. Boake *et al.* (1998) have found codominance or polygenic inheritance in species discrimination of *D. heteronera* and *F₁* females against *D. silvestris* males by studying the probability of heterospecific copulation. There are, however, also examples of studies with some emphasis on female behaviour. Coyne (1993) has suggested that *D. simulans* females discriminate against *D. mauritiana* males, because they can detect differences in male genitalia. He found that the *D. simulans* females only seldom spread their wings when courted by *D. mauritiana* males and that they tried to dislodge the mounting males. Abnormally short between species copulations were found to be due to a species divergence at a minimum of three loci. In *D. mercatorum*, females have been found to express genetic variation in receptivity for males of different geographic origin (Ikeda *et al.* 1980). *D. mercatorum* females indicate their acceptance by spreading their wings, but Ikeda *et al.* (1980) did not make use of this signal.

### 3.4. Female receptivity versus species discrimination

The differences between *D. virilis* and *D. montana* females in species discrimination could not be documented directly, because both female types accepted *F₁* males, and because *D. virilis* males were reluctant or unable to court *D. montana* females (paper IV). We found, however, species differences in the consistency of the receptivity of females in within and between species courtship. The same *D. virilis* females were receptive in courtships with conspecific males and with *F₁* males (consistency in female
wing spreading and correlation in female acceptance speed). This holds true in *D. melanogaster* as well, where receptivity of the females in within species courtship and species discrimination against *D. simulans* males have been suggested to be parts of the same process (Carracedo *et al.* 1991, Izquierdo *et al.* 1992, Pineiro *et al.* 1993). Heisler (1984a) has also suggested that in *D. melanogaster* female discrimination against yellow males depends, in part, on the overall receptivity of the females.

We found no consistency in *D. montana* female wing spreading, and none of the female receptivity traits were correlated in courtships of these females with *D. montana* and F₁ males (paper IV). In *D. montana*, the traits affecting discrimination against F₁ males are evidently inherited independently from the traits affecting the receptivity of the females with conspecific males. Species discrimination and female receptivity seem to be inherited independently also in *D. mojavensis*. Koepfer (1987) has found that reduction in matings between geographic forms of this species due to artificial selection does not affect female mating propensity.

Why is there a tight connection between female receptivity with conspecific and heterospecific males in some species and not in the others? Both *D. virilis* and *D. melanogaster* are widely distributed human commensals and females of these species may hybridise quite easily with the males of closely related species (Throchmorton 1982, Carracedo *et al.* 1991), contrary to *D. montana* and *D. mojavensis* females (Throchmorton 1982, Koepfer 1987). *D. virilis* expresses many primitive features of *D. virilis* group (Throchmorton 1982), and *D. melanogaster* is regarded as the most primitive species in the *melanogaster* complex (Lachaise *et al.* 1988). *D. montana*, on the other hand, has been suggested to be possibly the most derived species of the *virilis* group (Throchmorton 1982, Spicer 1992). According to Kaneshiro (1976) and Ohta (1978), the females of derived species are not so critical in their mate choice as the females of ancestral species, which can indicate the direction of species evolution among Hawaiian *Drosophila* species. Even though Kaneshiro’s model may work under certain conditions, it fails to do that under others (Wasserman & Koepfer 1980). Watanabe and Kawanishi (1979) have presented an alternative model, in which the females of the derived species are supposed to be more discriminating than the females of the ancestral species. This would be the case in *D. virilis* group, where “derived” *D. montana* females exercise stronger species discrimination than “ancestral” *D. virilis* females. I think, however, that at this stage it may be premature to put much emphasis on phylogenetic differences in female species discrimination, in particular, as the division into ancestral and derived species seems to be highly subjective.

Signal traits (and female responses to these traits) may evolve rapidly and in unpredictable direction, and can result in rapid speciation at low levels of general genetic divergence (Butlin & Tregenza 1998). According to the authors this is especially true for species living in sympatry. In addition to selection against hybridisation, evolution of male trait *per se* (via sexual selection) may select for discriminating females (Holland & Rice 1998), which, as a by-product, do not accept heterospecific males.
3.5. Female requirement and predilection for male song

In most *Drosophila* species absence or presence of male courtship songs clearly affect the female willingness to mate (Bennet-Clark & Ewing 1969, Ikeda *et al.* 1981, Kyriacou & Hall 1982, Gailey *et al.* 1986, Crossley & Bennet-Clark 1993). The importance of male song for the females and the inheritance of female song requirement was studied in paper IV by observing the behavior of *D. virilis*, *D. montana*, F₁ and BC₉₀ females with winged and wingless F₁ hybrid males. *D. virilis* females occasionally spread their wings when courted by a wingless male, but the mating propensity and the acceptance speed of the females was lower when the female was courted by a wingless male than when she was courted by a winged male. Accordingly, *D. virilis* females have a song predilection, but do not require courtship song. On the contrary, none of *D. montana*, BC₉₀ and F₁ females spread their wings in courtship with a wingless male. *D. montana* and BC₉₀ females also tried to dislodge wingless males attempting ‘forced’ copulation. The observation that all F₁ and BC₉₀ hybrid females required male song suggests that genes for song requirement are dominant or hypostatic to *D. virilis* non-requirement genes or that song requirement is a threshold trait with a polygenic background.

What is the association between female receptivity and female requirement for male song? *D. virilis* females, which accepted a wingless F₁ male, differed from the ones which did not do this in their acceptance speed in within species courtship (paper IV). This suggests that in *D. virilis* females’ acceptance of mute males (no song requirement) may be explained by a high receptivity of the females. F₁ and BC₉₀ hybrid females resembled *D. montana* females in their song requirement, but not in their receptivity, i.e. species difference in female song requirement and female receptivity were inherited, at least in part, independently. Accordingly, it seems that the song requirement of *D. montana* females is a genetically determined character *per se*, and not only a consequence of the low receptivity of these females. Independent inheritance of the two traits could be verified by observing the behaviour of BC₉₀ females, but these progenies are difficult to obtain.

Importance of specific male signals for the females has been studied in *D. melanogaster*. The females of this species do not seem to have a requirement for any specific signal. Markow (1987) has, however, found that both sequential and quantitative properties of *D. melanogaster* courtship are altered when one of the courtship partners is deficient in his/her ability to receive certain sensory information. The same phenomenon has been observed also by Gailey *et al.* (1986) who have shown that the females of an olfactory-deficient mutant strain of *D. melanogaster* (olfD) have a low mating propensity, because the females are defective in their stopping behaviour. Also, Ewing (1964) and Burnet *et al.* (1971) have shown that hearing the male song lowers the amount of courtship the female requires from the male.

Selection experiments have confirmed that male song plays a role in *D. melanogaster* courtship. Cook (1973a, b) found an increase in the overall receptivity of *D. melanogaster* females (through reduction in locomotion) in selection experiments using males with partly amputated wings. McRobert *et al.* (1995) found increased female receptivity also in raised stocks of *D. melanogaster*. The wings of flies of this stock are locked in a vertical position making the males mute. Females of these stocks are more
receptive than wild-type females regardless of the type of courting male. This implies genetic variation in acceptance threshold for mute males in *D. melanogaster*. Through a genetic analysis McRobert *et al.* (1995) found the altered receptivity of females of raised stocks to be affected by dominant factors on the X and the third chromosome. Evidently, active or passive selection for increased receptivity of females with mute males in *D. melanogaster* had affected the female overall receptivity, lowering the acceptance threshold of the females. This agrees with the idea of close relationship between low receptivity and lack of song requirement, like in *D. virilis*. Selection did not apparently affect song predilection of *D. melanogaster* females as these females still preferred wild type males over the males of their own type.

*D. subobscura* and *D. montana* females require different courtship signals. The genetic basis of this requirement has been studied by selection experiments. *D. subobscura* flies require visual stimuli to perform courtship rituals including a lot of dancing (e.g. Spieth 1952). When a *D. subobscura* female is receptive, she stops dancing allowing the male to mount her. According to Pinsker (1980), light dependence of mating in this species is caused by male requirement for visual signals, because even blind females (uncapable to receive visual signals and perform dance rituals) may be inseminated. However, the reason for the insemination of the blind females may well have been that the males do not require female dance or female acceptance signal (stopping the dance) to attempt copulation, i.e. the males succeed to copulate without female acceptance. Selection for light-independent mating produced a strain, where the males rape the females without a preceding courtship (Pinsker 1980). Behavioural differences between the “light-independent” stock and wild strains are based on polygenic factors involving all four autosomes, but not the X-chromosome.

In *D. montana*, only a very small proportion of females is inseminated by wingless males, even if the females are kept with these males in a no-choice situation for several days (Aspi 1992). The proportion of mated females can, however, be increased by selection. On the basis of selection response, the heritability of the proportion of inseminated females with wingless males has been estimated to be rather high (0.518±0.243; Aspi 1992). It is, however, not known whether this increase is caused by an increased tendency of the males to copulate with the female without her acceptance (forced copulations), or by a change in female sexual behaviour, in particular in receptivity and/or song requirement. The definition of requirement implies that there is no phenotypic or genotypic variation in the importance of courtship signals within the species. If there is no variation in female signal requirement, there may be a strong selection pressure for the males with a deficient signal capacity to attempt forced copulations.

An important question in the study of female requirement and predilection for male signal is whether the signal has to be species-specific. The perception of species-specific components of male’s song may provide the female information on the species, genetic quality and condition of the courting male. Oguma *et al.* (1996) have found strong sexual isolation between *D. triauraria* and *D. auraria* in dark. Males of these species attempt to copulate with both types of females, but only hearing conspecific song provokes the females to spread their wings. This leads to copulation. In *D. biauraria*, in particular interpulse intervals (IPI) in heterospecific male song have been found to elicit the female rejection signal (Tomaru *et al.* 1995). They suggest that a restricted accuracy
in species recognition in light in *D. biauraria* is caused by a decreased possibility of the females to avoid forced copulations.

According to the chase-away model (Holland & Rice 1998), males are sometimes constrained to retain older ineffectual ornaments, when these are needed to achieve the required threshold levels of stimulation in females. If *D. montana* females required a certain song character only to be within an acceptable range, song requirement could be an ‘ancient’ character. The fact that hearing male song increases the possibilities of the females to discriminate against heterospecific males, and also to exercise sexual selection based on male song traits, suggests, however, that song requirement is not an ineffectual relict in this species. An important question, raised by these studies, is how much the male song traits can vary to be efficient in species recognition and sexual selection.

### 3.6. Responsiveness of *D. virilis* females to species-specific simulated courtship song

The importance of different characters of courtship song for the female can be studied by playing the females simulated songs with varying characters. If female acceptance can be detected by a specific signal, these experiments can be done without the presence of males. In paper III, 58% of *D. virilis* females (N=100) spread their wings in at least one of the two song simulation sessions, when they heard a species-specific courtship song. Female wing spreading behaviour was consistent and the number of simulated songs (e.g. pulse trains), following which the females spread their wings, was repeatable in two simulation sessions. This shows that the female readiness to exhibit acceptance during song simulation was consistent within individuals.

The consistency of wing spreading response of *D. virilis* females (whether and when the females spread their wings) to species-specific simulated song suggests that the females differ from each other in receptivity (paper III). These females have also been found to accept the first courting male in selection experiments, regardless of what kind of a song he produces (Hoikkala, unpublished). These findings are consistent with a chase away model that states that if there is a lack of sexual selection for male signals, there is no selection for increased female resistance/discrimination (Holland & Rice 1989). Ikeda *et al.* (1981) have detected considerable variability in the threshold level of females for auditory signals also among three *D. mercatorum* strains. The rank of the strains in female responsiveness for male songs was exactly the reverse to that of female mating propensity measured through mass mating tests.

Bastock and Manning (1955) have introduced a nongenetic concept “female courtship summation” to explain differences between females in the amount of courtship the females require from the courting male before mating. Bastock and Manning (1955) argue that there is a negative relationship between the amount of stimulation provided by the male per unit time and the courtship duration in *D. melanogaster*. In addition to female summation, this correlation could be due to the circumstance that intensively courting males attempt copulation earlier than more passive males, and may achieve copulation, if the female has no control on mating decision. Robertson’s (1982)
experiments with D. melanogaster did not confirm the additive nature of courtship stimuli. Even he, however, found consistency in the amount of pulse song required by a female in two different courtships. In our studies (paper III) the amount and the quality of courtship stimuli (song) were standardised and yet the D. virilis females differed from each other in their responsiveness to song. According to the female courtship summation concept of Bastock and Manning (1955), variation between females would be caused through females gathering simulated songs in their short-lived memory and when a certain threshold is exceeded, the females spread their wings. This concept does not, however, explain variation between females in threshold level and how the quality of song influences the amount of songs the female needs to reach the threshold. It must also be remembered that the female responsiveness to simulated song can be affected, in addition to female receptivity, also by female song preferences. As shown in paper III, the species-specific song may not be the most preferred song type for all females.

Most of the studies with simulated songs have been done by measuring copulations without regard to female acceptance or repelling signals, which makes it difficult to distinguish the effects of song on female and male behaviour (Bennet-Clark & Ewing 1969, von Schilcher 1976a,b, Crossley & Bennet-Clark 1993, Crossley et al. 1995, Greenacre et al. 1993). However, male courtship song or some characters of the song has been found to elicit female acceptance or repelling signals in D. mercatorum, D. auraria, D. biauraria (Ikeda et al. 1981, Oguma et al. 1996, Tomaru et al. 1995) and D. montana (Ritchie et al. 1998). The repeatability of female responses has not been measured in any of these studies. Simulated songs have also been found to affect the behaviour of courting males by increasing their locomotor activity and courtship interactions (von Schilcher 1976a, Crossley et al. 1995). The removal of male aristae did not completely abolish this effect (von Schilcher 1976b).

3.7. Female preferences on male song characters in D. virilis

Preferences of D. virilis females on male song characters were studied in paper III by playing the females simulated species-specific song and songs with modified sound pulses (PL) and/or interpulse interval (IPI), without the presence of male. The proportion of the females, which spread their wings when hearing species-specific song (45.1%, N=102), was over two times higher than that of the females responding to modified songs (song with long IPIs: 18.2%, N=99; song with both IPIs and PLs long: 20.0%, N=100). The majority of females responded to only one song type, which suggests that the preference windows of these females were narrowly rather than broadly tuned.

Female wing spreading response was affected only by the type of simulated song and not by the order of the songs played on successive days. In insects, contrary what may be the case with other animals, the effects of learning (and copying) are in general considered to affect very little the sexual drive of male and females (but see Kim & Ehrman 1998).

D. virilis females have not been found to exercise discrimination between conspecific males with small differences in song characters (Hoikkala, unpublished). Consequently,
we played these females modified songs with extremely large differences in given song traits (paper III). A majority of *D. virilis* females responded to a courtship song resembling that of the conspecific males. Some females, however, responded only to songs in which the IPI or PL was 10 SD longer than in the species-specific song. According to Widemo and Sæther (1999) this kind of variation between females in mating preferences can be adaptive. It is unlikely that the females responding to modified songs were more receptive than other females, because these females accepted even fewer song types that the rest of the females. Preference windows of the females may be quite large compared to variation found in the male trait in the wild (Ritchie *et al.* 1998). As a matter of fact, females of many species have been shown to prefer supernormal stimuli (studies reviewed by Ryan & Keddy-Hector 1992). *D. montana* females have also been found to prefer a courtship song frequency, which is outside the variation found in male songs in the wild (Ritchie, personal comment). Large preference windows at population level could be due to large preference windows of individual females, or the females may have narrow preference windows with differences between females in the mean preference value. Preference windows of *D. virilis* females seem to be of the last-mentioned type (paper III).

Modified IPI and PL in paper III were not within the range of variation found in the songs of *D. virilis* males. *D. virilis* females will hybridize in laboratory conditions with the males of several *D. virilis* group species, the songs of which differ even more than 10 SD in IPI and PL from the songs of conspecific males (Hoikkala & Lumme 1987). This suggests that song differences do not prevent these females from mating and some females may even be stimulated more by song type outside than that inside the range of variation within the species (paper III). As the repeatabilities of the female preferences for the modified songs were not studied, one cannot know how consistent the females were in their preferences.

Female preferences for simulated songs have been studied also in *D. montana* (Ritchie *et al.* 1998). Females of this species were played a simulated song that varied in pulse length and carrier frequency (song traits known to be targets of female choice in wild, Aspi & Hoikkala 1995). Female preferences were scored via the ‘wing spreading’ acceptance posture of females both alone and in the presence of mute males. In both situations, the females responded most readily to a song consisting of short sound pulses with a high carrier frequency. This confirms the findings of Aspi and Hoikkala (1995).

Models of sexual selection require that female preferences are heritable (e.g. Andersson 1994). There is, however, only limited evidence for genetic variation in female preferences (Bakker & Pomiankowski 1995). Ritchie (1991), Möller (1994), Wagner *et al.* (1995), Godin & Dudatkin (1995), Jennions *et al.* (1995) and Johnsen & Zuk (1996) have estimated repeatability of preference instead of heritability, which is a prerequisite for heritability in a behaviour pattern (Boake 1989). Most models on the evolution of exaggerated male traits (e.g. Fisher 1958, Zahavi 1977) also propose that female preference and male sexual trait have a correlated evolution. The probability that female preference modifies male trait depends, however, on the overall receptivity of the females, on the importance of the male signal for the females, on female sampling behaviour and on female control over copulation decision. These traits are more and less neglected by theoreticians and also by empirical researchers.

Whether the female chooses her mate according to her signal preferences depends
largely on her receptivity. In addition, the lack of clear acceptance and/or repelling signals may reduce the possibilities of the female to test and sample courting males, reducing the intensity of sexual selection within the species. In paper III, D. virilis females were found to vary in their responsiveness to simulated songs. The females of this species have, however, been found to be very receptive in intraspecific courtship and not to exercise mate choice on the basis of differences in male song (Hoikkala, unpublished). These females do not repel males attempting copulation without a preceding female wing spreading signal, either. The acceptance threshold of D. montana females is at much higher level than that of D. virilis females, enabling these females to choose their mates by comparing different males. D. montana females choose their mate on the basis of relative criteria (Hoikkala & Aspi 1995), which enables them to accept also low quality males in the absence of better males. The importance of a signal for the females per se (like the song requirement in D. montana) may promote more specific female preferences and male songs to evolve.

3.8. Repeatability of male song traits in D. montana and D. littoralis

D. montana and D. littoralis females use male courtship song in their mate choice in the wild preferring males that produce short and dense sound pulses (i.e. short PL and high CN and FRE, Aspi & Hoikkala 1995). In paper II these song characters were found to be repeatable among overwintered males of both species. Male progenies of wild-caught flies reared in the laboratory, and in D. montana also the males collected in the wild before overwintering, exhibited very little variation in the above-mentioned characters. In a fashion different from pulse characters, pulse train characters measured for D. montana song varied also between laboratory-reared males. Our findings suggest that in D. montana and D. littoralis, song characters that play a part in sexual selection in the wild (pulse characters) are more condition dependent than the song characters, which are not direct targets of female choice (pulse train characters). The repeatability of pulse characters also shows that the songs of overwintered males differ from each other sufficiently so that the females may exercise selection during the mating season.

Repeatability provides an indication of the potential utility of the signal as a reliable indicator of competitive ability of the males (Clark & Moore 1995). The high repeatability of pulse characters of the song among wild-caught D. littoralis and D. montana males was due to increased variation between males rather than to decreased variation within individuals (paper II). The fact that some pulse characters changed during the cold treatment suggests that they may carry information on the condition of an overwintered male, which enables the females to choose the males in good condition as a mating partner (Zahavi 1977). Characters of male acoustic signals are repeatable across a wide array of animal species, such as Chorthippus brunneus, a grasshopper (Butlin & Hewit 1986), Hyla versicolor, a tree frog (Gerhardt 1991), Nycticeius humeralis, a bat (Scherrer & Wilkinson 1993) and Grompadorhina portentos a cockroach (Clark & Moore 1995). We do not know, however, how large a proportion the variation between the songs of different males is due to changes in male condition.

The heritabilities of different song characters of D. montana and D. littoralis have
been estimated in the laboratory and in the wild (Aspi & Hoikkala 1993, Suvanto et al., in press) and in D. montana also before and after a cold-treatment in laboratory (Suvanto et al., in press). Aspi and Hoikkala (1993) found that in D. montana heritabilities calculated across the environments were nonsignificant (except for PN), while those for PN, IPI and PL in laboratory reared flies were significant. In our study (paper II) the songs of laboratory-reared D. montana males were found to be repeatable in PN and IPI but not in PL. The low repeatability of PL was unexpected, as repeatability should set an upper limit for the heritability of a character (Falconer 1989, Boake 1989).

In the study by Suvanto et al. (in press), most heritability estimates for D. montana song traits were nonsignificant, largely due to high residual variation. During the cold-treatment, however, the additive variation increased significantly in nearly all song traits, so that this increase was most pronounced in traits found to be the targets of sexual selection (PL, CN and FRE). The increase in additive variation was most probably due to the circumstance that the selected song traits became more repeatable following cold-treatment, i.e. to the same phenomenon, which we found in paper II.

One of the central questions in the theories of sexual selection is to what extent the development of male characters depends on the phenotypic condition and overall genotype of the male (Andersson 1986). In cases where Drosophila flies overwinter as adults and mate after overwintering (like in D. montana and in D. littoralis), the male trait could tell the female how well the male has survived the winter (Aspi et al. 1993). If genotype environment-interactions increase phenotypic variation between males in sexually selected song traits prior to the mating season of the flies, male songs may reflect viability and condition of the males at the moment of courtship. This is as suggested in conditional viability variants of the ‘good genes’ model (Zahavi 1977, Iwasa 1991). D. montana females that exercise selection on male song have also been found to gain indirect benefit from their choice in the form of better offspring survival (Hoikkala et al. 1998).
4. References


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