MATE CHOICE AND GENETIC VARIATION IN MALE COURTSHIP SONG IN DROSOPHILA MONTANA

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This thesis deals with factors affecting mate choice as well as with genetic variation in male courtship song in Drosophila montana. Males, which produced song with a high carrier frequency, were found to court females, and also to succeed in their courtship more often than the males producing low frequency song. Male mating success correlated with the carrier frequency of his song recorded after, but not before, an artificial winter, which suggests that a sexually selected male trait is sensitive to environmental factors. A high carrier frequency of male courtship song correlated positively with the survival rate of the males progeny from egg to adulthood (indirect benefit for the female), but not with the fecundity of his mating partner (no direct benefit for the female).

The heritabilities and the amount of additive and residual variation in male courtship song characters were measured in two populations using father-son regression and sib analysis. The songs of the males from one of these populations were analysed for a second time after the cold treatment. Most heritability values were insignificant, largely due to high residual variation. During the cold treatment, the additive variation increased and the residual variation decreased in almost all song traits. Increased variation in sexually selected traits may help the females to exercise selection between the males during the mating season of the flies in the wild in spring. This, and the fact that male song gives the female information about the male’s condition/genetic quality suggests that in this species the evolution of female preferences for male song characters could have evolved through condition-dependent viability selection as postulated by "good genes" models.

Variation and inbreeding depression/heterosis were studied in traits associated with fly reproduction using inbred D. montana strains. Songs, hydrocarbons and some behavioural traits of the flies varied significantly between strains. The strain of both sexes affected female egg-laying, and the female strain, also, the survival rate of the flies progeny, in different intra- and interspecific combinations. Heterosis was found in the mating propensity of the flies and in the carrier frequency of the male song. Diallel analysis revealed unidirectional dominance towards higher carrier frequency. This direction is the same as the direction of sexual selection exercised by the females of this species suggesting that sexual selection could be a driving force in evolution of this song trait.

Keywords: courtship signals, evolvability, female preference, sexual selection.
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Oulu, March 1999

Leena Suvanto
List of original papers

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

I  Hoikkala A & Suvanto L. Male courtship song frequency as an indicator of male mating success in *Drosophila montana*. Submitted for publication.


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1. Introduction

1.1. Courtship signals

Courtship in many animal species consists of a repertoire of specific signals delivered by the male and the female only during the mating event. These signals may play a part both in species recognition and in sexual selection within the species. In *Drosophila* species, signals delivered during the courtship can be acoustic, visual, tactile or chemical (Ewing 1983, Spieth & Ringo 1983, Spiess 1987).

The males of most *Drosophila* species produce acoustic signals, courtship songs, by vibrating their wings (Ewing 1983). These songs include sine song consisting of polycyclic humming sounds (resembling flight sound), and pulse song composed of trains of mono- or polycyclic sound pulses (Spiess 1987). In some species, male courtship songs may have features of both song types (Ewing & Miyan 1986). In *D. virilis* group, males of all species produce pulse-structured song as a primary courtship song (Hoikkala et al. 1982). In four species of this group, the males also produce secondary courtship song consisting of trains of sound pulses or of polycyclic hums (Suvanto et al. 1994). The importance of the song for male courtship success varies between species. In *D. montana* (a member of *D. virilis* group), the primary courtship song of the males seems to be the most important courtship signal. The females of this species need to hear the song of the courting male before they accept the male as their mating partner (Liimatainen et al. 1992).

In some *Drosophila* species, light is necessary for a successful courtship. For instance in *D. subobscura* and in most Hawaiian species, the flies need light to perform their courtship rituals successfully (Spiess 1987). Visual signals are not essential for the flies of *D. virilis* group species, since these flies can also mate in darkness (Hoikkala 1988).

Flies may receive tactile cues and/or pheromones when the male touches and licks the female. Fly pheromones, in the form of cuticular hydrocarbons, may be sex and species-specific (Cobb & Jallon 1990). For example, Coyne and Charlesworth (1997) have reported that sexual isolation between *D. sechellia* and *D. mauritiana* is partly caused by pheromonal differences between the two species. Bartelt et al. (1986) have analysed

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cuticular hydrocarbons of *D. virilis* group species. They found differences between sexes in five species of the group, but not in *D. montana*.

### 1.2. Sexual selection theories

The term “sexual selection” was first introduced in 1871 by Charles Darwin (1981). It means that the reproductive success of individuals varies due to mate choice targeted to specific secondary sexual traits. In several animal species males have conspicuous traits, which seem to be unnecessary or even handicaps for the males, but the females of the same species appear to favour them (Andersson 1982, Møller 1988, von Schantz *et al.* 1989, Hill 1991, Norris 1993, Petrie 1994, Hasselquist *et al.* 1996). All sexual selection theories presume that the female gets some kind of benefit from her mate choice. This benefit can either be direct i.e. benefit for the female herself or indirect i.e. benefit for her offspring. Direct benefits include nourishment, better fecundity, protection, avoidance of parasites and help in offspring raising (von Schantz *et al.* 1989, Hill 1991, Moore 1994). Indirect benefits for the female are e.g. a better survival rate (from egg to adulthood) and a longer life span of her offspring.

Three main theories with no direct benefits for the female have been evoked to explain the evolution of male traits and female preferences for them: “Fisherian runaway process” (Fisher 1915, 1958), “indicator model” (Zahavi 1975, 1977) and “sensory exploitation model” (e.g. Ryan & Rand 1990). Fisher introduced a theory, where the female preference for a specific male trait is genetically connected to this trait. Females choosing attractive males will have attractive sons, which are again favoured by the females. The phenomenon reinforces itself, when the females choose more and more exaggerated male traits and the males develop bigger handicaps. At the same time female choice also leads indirectly to a higher demand for the male trait, because of the positive genetic correlation between male trait and female preference for it. This goes on until the male handicap (and the female preference for it) reduces the fitness of the male (and female) too much, and the handicap is restricted by natural selection. An ultimate version of Fisher’s theory, “sexy son hypothesis”, was presented by Weatherhead & Robertson (1979). They suggested that females mating with attractive males will actually suffer from reduced reproduction success, but this will be compensated for by the high mating success of their sons. Fisher did not present any mathematical model for his theory, but such models have been introduced later, e.g. by Lande (1981) and Kirkpatrick (1982). Also, Heisler (1985) presented a model for the evolution of “arbitrary” mating preferences, i.e. preferences directed at characters that may not themselves be the cause of variation in fitness, but which may reflect that variation due to genetic correlations with directly selected characters.

The indicator model is based on an idea that the male’s trait gives the female information on the male's fitness. This model was first presented by Zahavi (1975, 1977). He suggested that the male’s handicap is a test of his condition. A male with a better level of fitness can produce a more extreme trait than a male with a lower level of fitness, supposing that the male trait is restricted by natural selection i.e. the trait is costly for the
male. The indicator model is also called the “handicap principle” and, according to some of its modified forms, the “good genes theory”. The good genes theory suggests that the male trait reflects the male’s overall genetic quality (Kodric-Brown & Brown 1984). One variation of the indicator theory is the parasite model of Hamilton & Zuk (1982). In this model sexually selected traits are expected to reveal information about the male’s resistance to parasites. The condition of male trait shows the female whether the male has (or has had) parasites, enabling the females to choose males, which are most resistant to parasites.

Sensory exploitation hypothesis (e.g. Ryan & Rand 1990) suggests that female preferences do not coevolve with male sexual signals. According to this model, the male trait is favoured simply by virtue of its manipulative effect on a pre-existing bias in the sensory system of females. Biases in female preferences evolve for reasons unrelated to sexual selection. Contrary to two models presented above, the male trait and female preference for the trait are not expected to have a tightly correlated evolution.

Empirical evidence for the validity of sexual selection theories is limited. Both Fisherian and indicator models require the existence of genetic variation in male trait and female preference, as well as genetic correlation between the two traits. The prime way to distinguish between these models is to look at the effect of mate choice on mating success (Fisherian model) and genetic quality (indicator model) of offspring. Most of the evidence supporting the indicator theory comes from studies tracing the consequences of mate choice on the fitness of the female’s offspring. Norris (1993) has reported that the black breast stripe in great tits (Parus major) is a sexually selected trait, and the males with the largest stripes have the best surviving offspring. In collared flycatchers (Ficedula albicollis) the size of the forehead patch of the male is found to contribute genetically to the fledging condition of the nestlings (Sheldon et al. 1997). House finch (Carpodacus mexicanus) females prefer bright coloured males, because they get resources and possibly also genetic benefits for the offspring (Hill 1991). In great reed warblers (Acrocephalus arundinaceus), females choose mates according to the territory quality and song repertoire of males. The post-fledging survival of the offspring is correlated with their father’s song repertoire (Hasselquist et al. 1996). Also, in Nauphoeta cinerea cockroaches (Moore 1994), Drosophila melanogaster (Partridge 1980), Pavo cristatus peacocks (Petrie 1994) and Hyla versicolor gray tree frogs (Welch et al. 1998), offspring fitness has been found to increase, if the female can choose her mate.

There are also a few examples supporting the sensory exploitation theory. Male calls in Physalaemus pustulosus frogs seem to have evolved towards a low carrier frequency, preferred by the females. This seems to be because the structure of female’s ear is more sensitive to low than high frequency. Also the females of a related species, P. coloradorum, have this preference, but the males of this species cannot produce lower frequency “chucks”. This suggests that the preference for low frequency calls already existed in females before the chucks evolved in males (Ryan et al. 1990). Basolo (1990) has studied genus Xiphophorus, which consists of swordless platyfish and swordtails. In this genus, swordlessness is the primitive state. In platyfishes (Xiphophorus maculatus), the females have been found to prefer conspecific males with artificial swords over those without swords, despite evidence that the common ancestor of platyfish and swordtails was swordless (Basolo 1990). In water mites (Neumania papillator), the courting males
vibrate their legs fanning pheromones towards the females. Females respond to this vibration as they do to moving prey, and recognise the males as potential mates only after the males have deposited spermatophores. It seems that male vibration has evolved as a response for female movements (Proctor 1991).

1.3. Variation in sexually selected traits

1.3.1 Heritability of fitness traits

Heritability ($h^2$) is the ratio between additive genetic variance ($V_A$) of a trait and its total phenotypic variance ($V_P$). Heritability measures the degree of resemblance between relatives, and it also determines how the trait responds to selection (the amount of $V_A$). A fitness character with a high heritability evolves rapidly, which may lead to fixation of beneficial alleles and to a reduction in genetic variation (Falconer & Mackay 1996). Falconer and Mackay (1996) stated that traits closely related to fitness have lower heritabilities than the traits more distantly related to fitness. This claim is supported by the experimental studies of Gustafsson (1986) in collared flycatchers (Ficedula albicollis) and Mühlhäuser et al. (1996) in yellow dung flies (Scathophaga stercoraria), as well as by the studies reviewed by Mousseau and Roff (1987) and Roff and Mousseau (1987).

The amount of additive variation in a specific trait may be high enough for the trait to evolve further, even though its heritability would be low. Low heritability values may be caused, in addition to low additive variation, also by high residual variation (Houle 1992, Campbell 1997). Likewise, there is ample experimental evidence to suggest that the amount of environmental and genetic variance can vary depending on the environmental conditions, such as stress (Hoffmann & Schiffer 1998, Sgrò & Hoffmann 1998, Hoffmann & Merilä 1999). It must be remembered that the heritability value of a given trait always refers to a specific population under specific conditions (Falconer & Mackay 1996). When comparing heritabilities, which have been measured in different populations, special attention must be paid to the environment, population structure and measurement techniques (Falconer & Mackay 1996). According to Houle (1992), coefficients of variation are less susceptible to variation in experimental design and also more useful in comparisons of traits differing in their mean values.

1.3.2. The concept of lek paradox

In species where the males do not offer the female any direct benefits such as protection, food or paternal care, there does not seem to be much reason for the females to choose their mate. However, also in these species the females have been shown to exercise mate choice, preferring males with specific characters (Andersson 1994). This may lead to the
exhaustion of genetic variation in male traits, which leaves the females nothing to select from. The phenomenon has been called "the lek paradox", because many (but not all) of the species exercising mate choice without direct benefits are lekking species. There has been a lot of debate on the existence of the lek paradox. It has been suggested that the females get direct benefits, such as increased fertilisation success (Bourne 1993) or reduction in mate searching costs (Grafe 1997), from their mate choice, even though these benefits may have remained undetected. Sexual selection in lekking species may also not be as strong as has usually been thought (Lanctot et al. 1997), or the costs of mate choice for the females may be very low leading to high net benefits (Reynolds & Gross 1990).

Pomiankowski and Møller (1995) have suggested that a reasonable amount of additive variation can be preserved in sexual traits under long-term directional selection, if this selection is greater than linear, and if it favours modifier genes increasing phenotypic variation in the trait. Their compilation of data suggests that additive variation in sexually selected male traits may be even higher than variation in the same traits in females or in non-sexually selected traits in males. Rowe and Houle (1996) suggest that sexually selected traits are condition dependent, and that there is a high genetic variation in male condition. According to this model, evolution of condition dependence in traits under selection will lead directly to an increase in the genetic variance, in those traits. The main difference between Pomiankowski-Møller and Rowe-Houle models is that the latter model does not require selection on variance of sexually selected traits.

1.3.3. The maintenance of genetic variation

Several mechanisms have been suggested to maintain additive genetic variation in populations (Roff 1997). In mutation-selection balance theory, input of variation through new mutations is expected to balance the loss of variation caused by stabilising selection, thereby preserving the variation. Mathematical models of this theory have been presented e.g. by Lande (1976), Turelli (1984), Rice (1988), Bulmer (1989), Houle (1991) and Keightley (1991). According to Roff (1997), mutation-selection balance may, however, not be powerful enough in retaining variation in fitness traits which are under strong selection pressure. Roff (1997) suggests heterozygote advantage to be one obvious candidate for preserving genetic variation in these kind of traits. There is, however, no experimental evidence to support this, and theoretical studies suggest that it is an unlikely mechanism to maintain genetic variation. Antagonistic pleiotropy means that the same gene can affect e.g. two different fitness traits, strengthening the impact of one trait while at the same time it diminishes the other trait. There is evidence suggesting both that this mechanism plays an important role in maintaining genetic variation (the length of the larval period in wood frogs, *Rana sylvatica*, Berven & Gill 1983), or does not play a role in it (male life-history traits in *Drosophila*, Hughes 1995). Curtisinger et al. (1994) have suggested that from a theoretical point of view antagonistic pleiotropy does not seem to be a likely mechanism to maintain variation, but there are also opposite estimations (Roff 1997). Other important factors suggested to preserve variation in populations are e.g.

1.3.4. Fluctuating asymmetry as an indicator of male quality

Fluctuating asymmetry (FA) is a very popular measure of developmental permanence of bilaterally symmetrical traits (Palmer & Strobeck 1986). FA can be described as having a normal distribution of right minus left side differences, whose mean is zero (Palmer & Strobeck 1986). It has been suggested that FA is a good indicator of the sensitivity of a character to environmental and genetic stresses (Parsons 1992, Møller & Pomiankowski 1993). This is because both sides of the trait are constructed by the same genome and any asymmetry between sides reflects developmental confusion (Woods et al. 1998). Experimental evidence supporting this hypothesis has been presented e.g. by Møller (1996). He found that asymmetric domestic flies (Musca domestica) were more sensitive to predators and parasites than symmetric flies. Fluctuating asymmetry was also positively associated with the flies’ mating success (Møller 1996). Woods et al. (1998) agree that FA may be a good indicator of environmental conditions, but, at least in Drosophila, it may not be a reliable measure of genetic quality because of its low heritabilities.

1.4. Purpose of the present study

The aim of this thesis was to investigate female mate choice and the factors affecting it, as well as the genetic variation in male courtship song in Drosophila montana. In the first paper (I), we studied whether male courtship song traits, male behaviour or male size affects male mating success, and whether the selected male traits are sensitive to environmental factors. In paper II, our aim was to find out whether the females gain any direct or indirect benefits from their mate choice. Here, we studied the effects of male courtship song traits, size and possible asymmetries on female egg production and the fitness of her progeny. In paper III, we estimated heritabilities and the amount of additive genetic and residual variation in male song traits in two northern populations of D. montana. We also studied plasticity of male song traits, and changes in variance components of these traits due to environmental stress. In paper IV, we studied variation in fly mating signals and inbreeding depression/heterosis in traits associated with reproduction, using inbred D. montana strains. We first analysed the courtship songs of the males and the cuticular hydrocarbons of both sexes, of five inbred fly strains. We then studied the mating propensity and behaviour of the flies in different intra- and interstrain combinations, as well as the fecundity of the females and the survival rate of their progeny in each cross. Finally we examined the genetic basis of different male song traits, using an approach which allowed us to trace the direction of song evolution.
2. Materials and methods

The materials and methods used in these studies are described in detail in the original papers and only an outline is presented here.

2.1. Flies

*D. montana* flies were collected in spring 1996 from Kemi (65°40'N, 23°35'E) and from Oulanka, Kuusamo (66°22'N, 29°21'E), both situated in northern Finland. The flies were captured using malt baits. Females collected from wild were allowed to lay eggs in malt medium vials in the laboratory. Males collected from Kemi were studied in experiments described in paper II. F1 progenies of Kemi and Oulanka females were used in the studies described in paper III. F2 progenies of these females were used in experiments described in papers I and III.

In studies described in paper IV we used *D. montana* strains, which had been inbred by brother-sister matings for 20 generations. Three of these strains are from Finland: strains 1251 and O7 from Oulanka (maintained in the laboratory since 1981 and 1985, respectively) and K12 from Kemi (1985). Strain 1263 originated from Kawasaki (34°80’N, 139°42’E), Japan and strain 1550 from Yukon (61°30’N, 159°20’W), Alaska, USA.

The flies were maintained in a fly room in continuous light, at 19°C. Freshly emerged flies were sexed under light CO2 anaesthesia within three days of hatching. Males and females were kept separately in malt medium vials and used in song recordings, behaviour experiments and/or crosses when sexually mature (at the age of three to four weeks). In papers I and III a group of the flies were maintained in dark cold room (4°C) for six months in sugar-agar vials.
2.2. Song recording and analysis

All four studies included recording and analysing the male courtship songs. The songs were recorded in a special courtship chamber, which consisted of a Petri dish (diameter 5 cm, height 0.7 cm) covered with a nylon net. On the bottom of the chamber was a moistened filter paper. The songs of the males were recorded in a single pair courtship (with females from a multifemale *D. montana* stock), using a JVC-condenser microphone and a Sony TC-FX33 cassette recorder. The recordings were performed at 19±1°C.

The songs were analysed using SIGNAL Sound Analysis System (© Engineering Design). The traits analysed from songs were the length of the pulse train (PTL), the number of pulses in a pulse train (PN), the interpulse interval, i.e. the time from the beginning of a pulse to the beginning of the next pulse (IPI), the pulse length (PL), the number of cycles in a pulse (CN) and finally the carrier frequency of the song (FRE), using the Fourier spectra.

2.3. Behaviour studies

The mating behaviour of the flies was studied in papers I and IV. In paper I we studied female choice by placing one female and two males in the same courtship chamber. Each male was allowed to compete against all other males of the group. In paper I, the behavioural traits observed were; the mating success of the flies (defined as the number of trials where the male succeeded to mate with the female), the latency period (time from the beginning of the experiment to the first courtship act of the male), active courtship time (total length of the courtship bouts of the male directed towards the female) and the number of songs emitted by the male during the courtship. In paper IV, we measured the length of the latency period, mating speed of the flies (time from the beginning of the experiment to copulation) and copulation duration.

2.4. Morphological measurements

Morphological measurements were made for flies in papers I and II. In order to measure size variation between males, the width and the length of the left wing of each male was measured as described in paper I. We also measured possible asymmetries in wings in paper II. This procedure is described in detail in paper II.

2.5. Hydrocarbon analysis

Cuticular hydrocarbons of inbred *D. montana* strains were analysed in paper IV. Hydrocarbons were extracted using hexane and the samples were analysed with a gas
chromatograph (Varian 3400). 28 peaks were identified and the statistical analyses were done as described in paper IV.

2.6. Female egg-laying and the survival rate of the fly progenies

The number of eggs laid by the female, as well as the number of flies emerging from the pupae, was counted in papers II and IV. This was done in order to detect possible direct and indirect benefits the females could have gained from mating with a given male (paper II) and to discover possible heterosis in crosses between inbred *D. montana* strains (paper IV). The procedures are described in more detail in papers II and IV.

2.7. Statistics

The statistical analyses were performed using parametric tests (t-tests, ANOVA), when the data were normally distributed, or when it could be normalised by transformation. In other cases we used non-parametric tests (Kruskal-Wallis test).

Selection differentials and selection gradients were used to measure the connections between male song and male wing characters, female egg production, and the survival rate of the flies’ progeny, as well as between male wing characters and female egg production, and progeny survival (paper II). Selection differentials describe the net estimated selection and selection gradients the direct effect of selection on each trait (Roff 1997). They were estimated as described in paper II.

Repeatabilities of different song characters (papers I and III) and wing measurements (paper II) were estimated as described by Lessels and Boag (1987), and the standard errors of repeatabilities were calculated using a method described by Becker (1992). Narrow sense heritabilities (paper III) were calculated separately from sire and dam components as described by Falconer and Mackay (1996). The standard errors of heritabilities were calculated using a method described by Becker (1992). Coefficients of variation were calculated following Houle (1992).

Nested ANOVA, along with regression analysis, were used to measure the amount of additive genetic variation in the songs of full- and half-sib progenies from the Kemi and Oulanka populations (paper III). The differences in fly cuticular hydrocarbons between sexes and between inbred strains were studied with a multivariate analysis of variance (paper IV). A more detailed analysis was further done using a principal component analysis (paper IV).

Diallel analysis was done in order to study the magnitude of different variance components (additive and dominance variation) in *D. montana* song (paper IV). This analysis provides a test for the significance of additive and non-additive effects and for possible reciprocal differences among progeny families (Mather & Jinks 1982). The diallel data were analysed as described in paper IV following Hayman (1954a, 1954b) and Mather and Jinks (1982).
3. Results and discussion

3.1. Sexual selection in *D. montana*

3.1.1. Traits affecting male mating success

In the first paper we studied whether the size, behaviour or any of the song characters of the male affect male mating success. Among the song traits, only the carrier frequency of the song appeared to correlate with a male's mating success. Correlation was significant between male mating success and the carrier frequency of the songs recorded after the males had been kept in a cold-room for six months. It was not, however, significant between male mating success and the carrier frequency of the songs recorded before the cold-treatment, suggesting that the preferred song trait may change considerably due to environmental stress. Also, the male’s tendency to begin to court the female correlated with male mating success, as well as with the carrier frequency of his song recorded after the cold-treatment. None of the other behavioural traits of the males (latency to courtship, courtship activity, number of songs emitted during the courtship), nor the size of the males, correlated with male mating success.

Correlation between the carrier frequency of the male song and the tendency of the male to begin the courtship suggests that male song frequency may be an indicator of male’s sexual drive. The fact that males vibrating their wings at a high frequency achieved more matings than the males vibrating their wings at a lower frequency, could be due either to active or passive female choice. In active choice, the females actively choose between courting males, while in passive choice the outcome of the courtship depends largely on the male sexual drive and/or on male-male competition. In study I female preference for high frequency singers was apparent, both, when the female was courted by only one male and when she was courted by two males. Also, fights between the courting males were extremely rare. Consequently, females can be said to exercise active mate choice on the basis of male song frequency.

There are a few empirical studies where the females have been shown to choose their mating partner according to some external male trait. These traits include: plumage

Male size has been found to be a sexually selected trait in many *Drosophila* species. Aspi and Hoikkala (1995) have earlier found small *D. montana* males to succeed better than big males in achieving mating in the wild. This happened, however, only during one of the two study years. In the present study (paper I) we did not find male size to affect male mating success. The contradiction between the present and the earlier study owe to different conditions in the laboratory and in the wild. Male mating success can also be affected by his age (Long *et al.* 1980).

Most studies on *Drosophila* species have shown larger males to have better mating success than smaller males. These include studies on *D. buzzatii* (Santos *et al.* 1988), *D. melanogaster* (Partridge *et al.* 1987, Taylor & Kekic 1988), *D. mojavensis* (Markow & Ricker 1992), *D. simulans* (Markow & Ricker 1992) and *D. testacea* (James & Jaenike 1992). In *D. pseudoobscura*, the results are contradictory. Partridge *et al.* (1987) found larger males to have a higher mating success than smaller males, while Markow and Ricker (1992) could not detect any differences in the mating success of males of different sizes. A high mating success of large males is usually considered to be due to the superiority of these males in fights between males (Partridge & Farquhar 1983). Smaller males have been found to gain more matings than larger males in *D. subobscura* (Steele & Partridge 1988) and several other Diptera species (McLachlan & Allen 1987). The better mating success of smaller males may be due to their superior skill in courtship movements compared to larger males (McLachlan & Allen 1987, Steele & Partridge 1988). Hence, the targets of sexual selection vary in different species and our results suggest that male size is not important to female choice in *D. montana*.

### 3.1.2. Benefits of mate choice for the female

In paper II we studied possible direct and indirect benefits the female could gain when mating with a preferred male type. Direct benefits are the benefits for the female herself (e.g. nourishment and better fecundity), whereas indirect benefits profit the female’s offspring (e.g. better survival from egg to adult and longevity). We could not detect any direct benefits, which *D. montana* females could have gained from mating with a male with specific song characters, i.e. the number of eggs laid by the female did not correlate with any of the song traits of her mating partner. *D. montana* females were, however, found to gain indirect benefit when mating with the males producing song with a high carrier frequency. Selection differentials suggested that the males producing a high frequency song also had better progeny survival than the males producing song with a
lower frequency. Also, the selection gradient analysis suggested male song frequency to be the target of selection. A female could increase the viability of her offspring by 24%, when choosing a male whose song frequency was one standard deviation above the average in the population.

Kirkpatrick and Barton (1997) have suggested direct selection to be more important than indirect selection in the evolution of female preference genes. Some Drosophila females have been reported to get nourishment from males during the courtship as a direct benefit for female (Markow & Ankney 1984, Steele 1986). This does not occur in D. montana (Aspi 1992). Chapco and Ebisuzaki (1978) have reported that in D. melanogaster egg production is almost entirely maternally determined, but egg-to-adult survival is influenced both by maternal and non-maternal effects. This may be true also in D. montana, where paternal effects on egg-to-adult survival were emphasised (paper II). Indirect benefits from mate choice have earlier been reported among Drosophila species only in D. melanogaster, where mate choice has been found to increase offspring fitness (Partridge 1980). Other reports of indirect benefits for the females are; faster offspring development in cockroaches Nauphoeta cinerea (Moore 1994), an increase in offspring viability in great tits (Parus major, Norris 1993), peacocks (Pavo cristatus, Petrie 1994), great reed warblers (Acrocephalus arundinaceus, Hasselquist et al. 1996) and collared flycatchers (Sheldon et al. 1997). When direct selection for preference genes is weak, indirect selection for preferences caused by good genes can be important in preference evolution and in sexual selection of male traits (Kirkpatrick 1996).

3.1.3. Fluctuating asymmetry as an indicator of male quality

Fluctuating asymmetry has been proposed to be a measure of male quality, because it reflects the sensitivity of an individual to environmental and genetic stresses (Parsons 1992, Møller & Pomiankowski 1993). Møller and Pomiankowski (1993) have found more FA in secondary sexual traits than in other morphological traits. Furthermore, wing symmetry has been suggested to be important to D. pseudoobscura, because asymmetry may disturb signalling between male and female (Markow & Ricker 1992). We could not find any correlations between male wing FA and song traits, male wing FA and female fecundity or male wing FA and offspring viability in D. montana. However, earlier studies on fluctuating asymmetry have also given very inconsistent results (Palmer & Strobeck 1986). Rowe et al. (1997) have suggested that FA is often mixed with antisymmetry, which might be a better indicator of condition than FA. The difference between these two asymmetry types is that fluctuating asymmetry has a normal distribution of right-minus-left (R-L) differences, while antisymmetry is distinguished by a platykurtic or bimodal distribution (Palmer & Strobeck 1986). Furthermore, Woods et al. (1998) have found in their studies on D. melanogaster bristle and wing traits that FA has a low heritability. They suggest that FA may therefore be an inferior indicator of genetic quality in Drosophila.
3.2. Variation in male song traits in *D. montana*

3.2.1. Genetic variation in male song traits in wild populations

Variation in male song traits was studied in Kemi and Oulanka populations in paper III. The songs of the F2 progenies of the females captured from these populations differed significantly from each other in all song parameters, except in the carrier frequency of the song. Males from the Kemi population had higher pulse and cycle numbers and longer sound pulses, interpulse intervals and pulse trains than the males from the Oulanka population (Table 3 in paper IV). The heritabilities of male song traits were highly variable in both populations, and they also had large standard errors. The only significant heritability, $h^2 = 0.55$, was the heritability of the carrier frequency estimated by sib analysis in the Oulanka population. Heritability of this song trait was even higher in the Kemi population ($h^2 = 0.59$), but due to high standard error it remained nonsignificant. Coefficients of additive variation varied depending on the studied trait, the method used for estimating genetic parameters (regression or sib analysis) and the population, where the flies came from. In the Kemi population, the sib analysis gave higher CV_A for half of the song traits than regression analysis, the results being partly contradictory. In the Oulanka population, the amount of additive variation was very low in most song traits in both analyses. Variation in heritability estimates, obtained with different techniques, has also been observed in earlier studies (Roff & Mousseau 1987). Since almost all of our heritability estimates were nonsignificant, the comparison of the reliability of different techniques is useless.

Falconer (1981) has suggested that the traits closely related to fitness have lower heritabilities than the traits which are more distantly related to fitness. This has been confirmed e.g. by Roff and Mousseau (1987), who reviewed literature for heritabilities of different traits (classified as morphological, behavioural, physiological and life history characters) in *Drosophila*. However, Houle (1992) has shown that although $h^2$ values are typically smaller for life-history characters than for morphological traits, the relationship is reverse, if the coefficients of additive variation are compared. In *D. montana* song, pulse characters can be regarded as fitness characters, because the females of this species select their mates according to these characters (Aspi & Hoikkala 1995, Ritchie et al. 1998, paper I), and because the females get indirect benefit from their choice (paper II). In our study, heritabilities were nonsignificant for most song traits in both study populations. The amount of additive variation (measured as a coefficient of additive variation) was at the same level, or even higher, in pulse characters as in pulse train characters, which gives support to Houle’s (1992) suggestion of a higher level of additive variation in fitness than in non-fitness traits.

Heritabilities of song characters have been studied earlier in *D. montana* Kemi population by Aspi and Hoikkala (1993), using father-son regression between wild-caught fathers and their laboratory-reared sons, and between fathers and sons reared in the laboratory. In their study, the heritability estimates across environments were low and nonsignificant, while for laboratory reared flies they were significant in some song traits (PN, IPI and PL). The difference between the results of the present study and the study by
Aspi and Hoikkala (1993) may largely be due to different measuring techniques. Aspi and Hoikkala calculated means of song characters over five pulse trains of each male, while we analysed only one pulse train per male. We chose to use the latter technique, because we wanted to reveal the whole amount of residual variation in male songs, including variation within the males. Heritability has usually been estimated by minimising environmental variance, because high residual variation easily leads to nonsignificant heritability. This kind of study technique systematically inflates h².

The amount of additive variation, measured as a coefficient of variation, was very low in most song traits of the males from the Oulanka population. Sib analysis revealed additive variation only in cycle number and carrier frequency in the Oulanka population, while it revealed additive variation in all song traits in the Kemi population. The difference in the amount of additive variation in the two populations may be due to different selection pressures or to differences in mechanisms maintaining genetic variation in populations. In Oulanka, the mating season of the flies is a few weeks later than in the Kemi population. The two populations also differ in their species constitution. In Kemi, the fly population on our study site consists of four D. virilis group species: D. littoralis, D. montana, D. lummei and D. ezoana, D. littoralis being the most abundant species (Aspi et al. 1993). In Oulanka, the riverside population consists of mainly two D. virilis group species, D. montana and D. ezoana, with about equal frequencies (Lumme et al. 1979). Among the Finnish D. virilis group species, male courtship song is an essential part of the courtship in D. montana and D. ezoana. Mate choice in D. montana has so far been studied only in the Kemi population, where the females have been found to prefer males producing short and dense (i.e. high frequency) sound pulses (Aspi & Hoikkala 1995). In this population, interspecific courtship has been found to usually break off, when the male begins to produce courtship song (Liimatainen & Hoikkala 1998). Differences between the songs of the males from the Kemi and Oulanka populations are most evident in pulse train characters, which may be more important in species’ recognition than in sexual selection. These characters vary distinctly between the species found in both of our study areas.

3.2.2. Plasticity of the song characters

Phenotypic plasticity is determined as the expressed phenotype of a genotype as a function of the environment (e.g. Scheiner 1993). It is usually restricted to the change that happens during the development of an individual. We applied the term plasticity to the changes in phenotype caused by the environmental factors during adult life.

In paper II, we exposed the males from the Oulanka population to an artificial winter (4°C for six months), and studied whether the songs of the males had changed during this treatment. Only the pulse number and pulse train length were repeatable over the recordings. The carrier frequency of the song differed between the recordings of the same males before and after cold treatment. Also, the mean frequency of male songs had increased during the treatment. A two-way ANOVA on half-sister progenies revealed a significant cold treatment effect in this trait. Interaction between the genotype and the
treatment appeared to be significant only in cycle number, i.e. the progenies having the highest cycle number before the cold treatment were different from those having the highest cycle number after the treatment. CV\textsubscript{A}s of all song traits, except interpulse interval, increased significantly and the CV\textsubscript{R}s of the same traits decreased during the cold treatment.

*D. montana* flies overwinter as adults and have a mating season in spring after the cold period (Aspi et al. 1993). During this season, the females exercise mate choice on pulse characters of the male song (Aspi & Hoikkala 1995). In study I, the females were also found to exercise the same kind of mate choice in the laboratory, among the cold-treated males. In study III, environmental factors, such as cold-treatment, were found to increase phenotypic variation in sexually selected song traits, revealing the additive component of variation. These findings suggest that the preferred male song traits are condition dependent and that they also vary genetically. There is a great deal of empirical support for the condition dependence of sexually selected male traits also in other species (Andersson 1994).

Can our findings be applied to the situation in wild populations? First of all, the conditions in the wild in winter are harder and more variable than they were in our cold-treatment experiment. This may lead to even higher CV\textsubscript{R} and nonsignificant heritabilities in male song traits in the wild (Aspi & Hoikkala 1993). Secondly, the carrier frequency of the male song is very sensitive to temperature changes, raising about 10 Hz per 1°C (Hoikkala 1985a). As the temperature can vary during the mating season of the flies about 10 degrees, the females have to be able to compensate for changes in male song frequency. An alternative is that the females simply choose the best singing male (Hoikkala & Aspi 1993).

### 3.3. Mate choice in *D. montana* in regard to sexual selection theories

Our findings show that *D. montana* females get an indirect benefit when they mate with attractive males. In our study, male song frequency was shown to be an indicator of the male’s quality. This finding supports the indicator model, which suggests that a male’s trait reflects his fitness. Song frequency was also shown to be sensitive to environmental stress. This is a requirement of the conditional variant of the good genes model. One assumption of the indicator model is that males cannot cheat when they advertise themselves. This means that sexually selected traits must be costly to the male i.e. its development is restricted if the male’s fitness is poor. We did not study the costs of singing for the male. However, the carrier frequency of the song is determined by the number of wing beats per second. Therefore, producing a high frequency song may be energetically demanding for the male.

Good genes models of the indicator theory suggest that the females choose their mate for indirect benefits. There are several studies supporting this claim (reviewed in chapter 3.1.2.). Also Fisher’s theory of arbitrary traits stresses the role of indirect benefits: in this theory, the female’s attraction to a sexually selected male trait is suggested to lead to better mating success of her sons among females having the same preferences. The good
genes model and arbitrary traits model make parallel predictions, because better mating success of ‘sons’ can also be due to good genes. Our findings in *D. montana* (papers I and II) support indicator theory (better survival of the progeny of males with preferred characters), but they do not exclude the arbitrary traits model.

Our studies on the songs of the males of full- and half-sib progenies showed that the preferred song traits of *D. montana* males vary genetically, and that they also are condition-dependent. Rowe and Houle (1996) have argued that the evolution of condition dependence in traits under sexual selection will lead directly to an increase in the genetic variance in those traits. This argument rests on two assumptions; condition dependence of sexually selected traits and high genetic variance in condition. According to Rowe and Houle (1996), fulfilment of these assumptions would lead to the resolution of the lek paradox as there is no exhaustion of genetic variation in sexually selected traits.

### 3.4. Courtship behaviour and signals of inbred *D. montana* strains

Courtship behaviour and mate choice of *Drosophila* flies has been shown to be affected by pheromones emitted in cuticular hydrocarbons by both sexes and by songs produced by the courting male (studies reviewed by Spiess 1987). In paper IV, we studied intraspecific variation in these traits with the aid of inbred *D. montana* strains originated from different areas of the species distribution. In the same paper we also studied inbreeding depression/heterosis in traits associated with fly reproduction, as well as the genetic basis of male song traits.

#### 3.4.1. Cuticular hydrocarbons

Cuticular hydrocarbons of both the males and the females varied between inbred *D. montana* strains (Fig. 1 in paper IV). Also, the two sexes differed from each other in their hydrocarbon composition. Variation between sexes was, however, independent from variation between strains. The strains from Japan and the USA differed from each other distinctly in male pheromones, the Finnish strains forming an intermediate group between these two extremes. Female pheromones could not be separated, according to their origin, so clearly.

Bartelt *et al.* (1986) have not detected any major differences between the cuticular hydrocarbons of different strains or sexes in *D. montana*. This is in contradiction to our results. The strains used in our study were from different parts of the world while in the study of Bartelt *et al.* they originated from only one continent. Also, the methods used by Bartelt and his co-workers (10-50 flies per sample, 24 h hexane extraction) differed from the methods used by us (1 fly per sample, 5 min hexane extraction). Toolson and Kuper-Simbrón (1989) have shown in *D. pseudoobscura* that long maintenance of the strains in laboratory conditions can lead to significant changes in cuticular hydrocarbon compositions. This may also have increased variation between our strains, which, in
addition to long laboratory maintenance, have been inbred for several generations.

3.4.2. Courtship behaviour of the flies

The flies from the inbred *D. montana* strains differed from each other in their mating propensity, i.e. in the proportion of flies mating during the observation period (paper IV). Other behavioural traits of the flies (latency, mating speed and copulation duration) did not vary between strains. The strain of both sexes significantly affected the mating propensity of flies. The female strain also affected the length of the latency period and the copulation duration.

Cuticular hydrocarbons have been shown to act as mating pheromones in many insect species, and even small changes in their composition have been found to affect mating success (Markow & Toolson 1990, Cobb & Jallon 1990). We found that in *D. montana* the latency period was determined by the females (table 3 in paper IV). This may be due to the females varying in their attractiveness because of their different cuticular hydrocarbon compositions.

Songs of inbred *D. montana* strains differed from each other especially in the number of pulses in a train and in the carrier frequency (Fig. 2 in paper IV). The fact, that the strain of the male significantly affected the mating propensity of the flies may not, however, have been due to male songs – at least the mean carrier frequency of the male song did not correlate with the mating success of the males of the studied strain. The songs of the males of all inbred strains had, however, a very low carrier frequency, and so their songs may not have been very stimulating for the females.

Mating propensity of *F*1 hybrid flies was higher than that of the flies of the parent generation, which refers to heterosis in this trait (table 2 in paper IV). The mating propensity of the flies has also been found to display directional dominance towards faster mating in several other studies (Collins & Hewitt 1984, Casares *et al.* 1993). In *D. melanogaster*, inbreeding has been found to affect both the mating activity and mate choice of the flies. This is suggested to be due to the flies being more attracted to genetically dissimilar mates and would therefore select mates that have pheromones different from their own. ("negative assortative mating"; Averhoff & Richardson 1974). The results of the present study (paper IV) do not confirm this phenomenon, as our flies mated as actively with the flies from their own strain as with those from alien strains, despite the fact that the hydrocarbon profiles of the flies from different strains differed from each other. Also other researchers have reported results that do not support negative assortative mating (Powell & Morton 1979, Veuille & Mazeau 1988).

3.4.3. Female egg-laying and the survival of the progenies

In *D. melanogaster*, female egg production is almost entirely maternally determined, whereas, the survival rate of the progeny is determined by both maternal and nonmaternal
effects (Chapco & Ebisuzaki 1978). In paper I, we had found the frequency of the male song to correlate with the survival rate of his progeny. In paper IV this kind of correlation was not found. Instead, we found that both the female and the male strain affected female egg-laying capacity. As Table 4 in paper IV shows, females mating with the males from one inbred strain laid a lot less eggs than the females mating with the males from the rest of the strains. The reason for this is unknown.

Females from inbred lines laid fewer eggs during the three days’ experiment (mean 27 ± 19, paper IV) than the females of *D. montana* multifemale stock mated with wild-caught males (mean 57 ± 21, paper II). The mean proportion of offspring surviving from eggs to adulthood was 0.31 ± 0.32 among the first mentioned females (paper IV), whereas among the latter females it was 0.52 ± 0.27 (paper II). This suggests inbreeding depression in the inbred lines. We did not, however, find any heterosis either in female egg-laying or in egg-to-adult viability in paper IV. This was surprising, as inbred *Drosophila* strains have usually been found to suffer from inbreeding depression and to show heterosis in strain crosses (Ehiobu et al. 1989, López-Fanjul & Villaverde 1989, García et al. 1994).

### 3.5. Genetic basis of male courtship song traits

Songs produced by the males from inbred *D. montana* strains differed clearly from each other (paper IV). A large variation between the songs of different strains could be due to variation within and/or between populations, to sexual selection during laboratory maintenance before inbreeding and to inbreeding itself. The carrier frequency of all inbred strains was much lower than that of wild caught males (papers II and IV), probably due to inbreeding depression.

The diallel analysis between inbred *D. montana* strains revealed significant variation between genotypes in all song traits, except in pulse length. We found additive variation both between arrays (i.e. progenies having one common parent) and between parental lines in pulse number, interpulse interval, cycle number and carrier frequency. Dominance was apparent in cycle number and carrier frequency. In cycle number it was caused by unequally distributed dominant alleles between the strains, while in frequency, the dominance was directional. *W/V* statistics, which can be used to analyse the direction of dominance, suggested that the alleles increasing the carrier frequency were dominant over the alleles decreasing the frequency. This suggests further that the directional dominance detected in carrier frequency was towards a higher carrier frequency.

The genetic basis of male song traits has been studied earlier with the aid of diallel analysis by Cowling (1980) and by Hoikkala and Lumme (1987). Cowling found that neither the interpulse interval nor the sine song frequency showed dominance, but that there was a significant additive component in both traits in *D. melanogaster*. Cowling suggested this to be due to the interpulse interval and sine song frequency not being important fitness characters in *D. melanogaster*. Hoikkala and Lumme (1987) studied the genetic basis of courtship songs in *D. virilis* phylad (Spicer 1992) from *D. virilis* group.
(D. a. americana, D. a. texana, D. novamexicana, D. lummei and D. virilis) with a diallel analysis. They found additive and dominance variance in all studied song traits (pulse train length, pulse number, interpulse interval, pulse length, cycle number and cycle length). Dominance was directional in all traits, except in pulse length. (Hoikkala & Lumme 1987.) The results of this analysis strongly suggest that the songs of D. virilis phylad species have been affected in past history by directional selection favouring longer pulse train length, higher pulse number and cycle number, and shorter interpulse interval and cycle length (i.e. higher carrier frequency). In species outside this phylad (including D. montana) the songs have evolved clearly species-specific.

Our diallel analysis revealed unidirectional dominance towards higher song frequency in D. montana. This is the same direction as the direction of female preference for male song from the same species (papers I and II). Sexual selection exercised by the females could therefore have been a driving force in song evolution towards higher carrier frequency. Male song traits, which have been found to differ most distinctly between D. montana and the rest of the species of D. virilis group (e.g. interpulse interval, Hoikkala 1985b) did not show directional dominance in diallel analysis. Consequently, female choice occurring presently in D. montana populations may not explain divergence of all male song traits. Directional selection for species-specific songs may well have operated during the divergence of these song traits, but this has not happened in the recent history of D. montana.
4. References


