JYVÄSKYLÄ 2004

Mikael Puurtinen

Evolution of Hermaphroditic Mating Systems in Animals

Mikael Puurtinen

Evolution of Hermaphroditic Mating Systems in Animals

Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Ambiotica-rakennuksen salissa (YAA303)
syyskuun 4. päivänä 2004 kello 12.

Academic dissertation to be publicly discussed, by permission of
the the Faculty of Mathematics and Science of the University of Jyväskylä,
in the Building Ambiotica, Auditorium YAA303, on September 4, 2004 at 12 o'clock noon.

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2004

Evolution of Hermaphroditic Mating Systems in Animals

Mikael Puurtinen

Evolution of Hermaphroditic Mating Systems in Animals

ABSTRACT

This thesis is focused on the advantages of hermaphroditism versus separate sexes in animals, on the evolution of self- and cross-fertilization in populations of the hermaphroditic aquatic snail *Lymnaea stagnalis*, and on the consequences of small historical population size for reproductive output and parasite resistance of snails. Hermaphroditism has been suggested to be favoured when the availability of mating partners is limited. Many animals can however increase the availability of mating partners by searching. We found that limited mate search efficiency stabilizes hermaphroditism, and more interestingly, that high mate search efficiency causes disruptive selection on searching behaviour, leading to the evolution of males and females. In our empirical studies of *Lymnaea stagnalis* populations, we found that these snails typically reproduce by cross-fertilization in nature. In experimental studies we found that evolution of self-fertilization does not seem to be opposed by lower fecundity of self-fertilizing snails, nor by considerably higher early mortality or higher risk of parasitism for self-fertilized offspring. Population genetic data revealed that the studied *Lymnaea stagnalis* populations had different histories that could be inferred from population genetic data; some populations had been very small and had lost most of the genetic variation, while others had retained larger sizes and more genetic variation. Small historical population sizes were found to be associated with slow maturation and low fecundity of snails, suggesting that slightly harmful mutations have accumulated in small populations by genetic drift, and that mutation accumulation may endanger the long-term persistence of small populations. Snails with low genetic variability were also more likely to become infected by trematode parasites, suggesting that loss of genetic variation may threaten the survival of small populations also on short time-scales.

Key words: Genetic drift; hermaphroditism; inbreeding depression; *Lymnaea stagnalis*; parasite resistance; population genetics; self-fertilization.

M. Puurtinen, University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, FI-40014 University of Jyväskylä, Finland

Author's address   Mikael Puurtinen
Department of Biological and Environmental Science
University of Jyväskylä
P.O. Box 35
FI-40014 University of Jyväskylä, Finland
e-mail: hemipu@cc.jyu.fi

Supervisor   Prof. Veijo Kaitala
Department of Ecology and Systematics
P.O. Box 7
FI-00014 University of Helsinki, Finland

Reviewers   Dos. Jukka Jokela
Department of Biology
P.O. Box 3000
FI-90014 University of Oulu, Finland

Prof. Hanna Kokko
Faculty of Biosciences
P.O. Box 56
FI-00014 University of Helsinki, Finland

Opponent   Dr. Pär K. Ingvarsson
Department of Ecology and Environmental Science
Umeå University
SE-90187 Umeå, Sweden

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which are referred to in the text by their Roman numerals. I have written all articles, except article II, which was written by K. Emily Knott. I have planned all studies, and done a large part of work in each study.

I   Puurtinen, M. & Kaitala, V. 2002. Mate-search efficiency can determine the evolution of separate sexes and the stability of hermaphroditism in animals. American Naturalist 160 (5), 645-660.

II   Knott, K. E., Puurtinen, M. & Kaitala, V. 2003. Primers for nine microsatellite loci in the hermaphroditic snail *Lymnaea stagnalis*. Molecular Ecology Notes 3, 333-335.

III   Puurtinen, M., Knott, K. E., Suonpää, S., Nissinen, K. & Kaitala, V. Can fitness effects of self- and cross-fertilization explain the natural mating system of the hermaphroditic snail *Lymnaea stagnalis*? Manuscript, submitted.

IV   Puurtinen, M., Hytönen, M., Knott, K. E., Taskinen, J., Nissinen, K. & Kaitala, V. The effects of mating system and genetic variability on susceptibility to trematode parasites in a freshwater snail, *Lymnaea stagnalis*. Manuscript, submitted.

V   Puurtinen, M., Knott, K. E., Suonpää, S., van Ooik, T. & Kaitala, V. 2004. Genetic variability and drift load in populations of an aquatic snail. Evolution 58 (4), 749-756.

1   INTRODUCTION

In this thesis I have studied the evolution of hermaphroditic mating systems in animals. Part of the work is purely theoretical, and deals with questions like what are the advantages and disadvantages of hermaphroditism compared to having separate males and females, and when does natural selection favour the evolution of either type of gender expression. Majority of the work however comprises empirical studies of ecology, population genetics, and mating system evolution in populations of the great pond snail, *Lymnaea stagnalis*. Because our study populations proved to be genetically isolated from each other, I was also able to study the effects of population size and isolation on reproductive output and parasite resistance of snails. These studies are relevant to the conservation of endangered species and populations, as human activities are increasingly leading to fragmentation of natural habitats causing declines in sizes and connectivity of natural populations.

1.1   Why be an animal hermaphrodite, and why not?

Hermaphrodites are individuals that possess both functional male and female reproductive systems. Hermaphroditism is typical for flowering plants, but it is also found in many groups of animals (Ghiselin 1969, Ghiselin 1974). The interesting question is why are some animals hermaphroditic, but most have separate males and females (i.e., are gonochoric). Answer to this question lays in the economics of resource allocation between different reproductive functions: if the overall fitness is greater for an individual that allocates resources to both male and female function than for an individual that only allocates to one function, hermaphroditism is favoured (Charnov et al. 1976). This statement is quite trivial, however. In order to understand the evolution of gender expression more precisely, we need to define those conditions that favour hermaphroditic sex-allocation strategies and those that favour specialization in male or female function.

Traditionally, evolutionary biologists have struggled to explain the existence of hermaphroditism in the Animal Kingdom rather than to explain

why most animals are gonochoric. A reason for this bias may be due to the fact that as humans we perceive having separate sexes as 'normal', whereas hermaphroditism seems 'odd'. The most influential theories for hermaphroditism relate to situations where availability of mating partners is low. Imagine a situation where each individual only meets one individual during its reproductive life. If the two individuals happened to be of the same sex they could not reproduce, but if both were hermaphroditic, cross-fertilization could take place (Tomlinson 1966, Ghiselin 1969). Most hermaphrodites are also able to reproduce without a partner by self-fertilization, an obvious advantage when mating partners cannot be found (Darwin 1876, Maynard Smith 1978). Further, if low availability of mating partners limits reproductive success through male function, a hermaphrodite may maximize its fitness by allocating most of its resources to female function (Altenburg 1934, Charnov et al. 1976).

Hermaphroditism thus seems to have several advantages over gonochorism, yet gonochorism is ubiquitous among animals. Obviously, there must exist some force that has led to the evolution and maintenance of separate sexes in the majority of animal taxa. This fundamental question has however received only minimal attention. Perhaps the most relevant contribution to this question has been an article by D. J. Heath (Heath 1977), where he showed that the extra cost of producing and maintaining both male and female reproductive apparatus would result in lower reproductive output of hermaphrodites compared to gonochorists. However, the extra cost of having both reproductive organs favours gonochorism only when allocation to male function is approximately equal to allocation to female function; hermaphroditism is stable even with the extra cost if sex-allocation is female-biased. While externally fertilizing hermaphroditic organisms may have an approximately equal sex-allocation, many internally fertilizing animal hermaphrodites probably do not. Also empirical studies trying to find evidence for existence of such costs have yielded equivocal results (Doums et al. 1998). It seems that current evolutionary theory cannot sufficiently explain one of the most prominent evolutionary phenomena: evolution and maintenance of separate sexes in animals.

1.2   Evolution of self- and cross-fertilization

Because most hermaphroditic organisms are able to reproduce by both self-and cross-fertilization, and the type of reproduction greatly influences the evolution and ecology of a species, it is of great interest to study the evolution of mating systems in hermaphrodites. Evolution of self-fertilization is facilitated by the intrinsic 3:2 transmission advantage of an allele for self-fertilization compared to an allele for cross-fertilization; an allele causing selfing can be present in two copies in every self-fertilized offspring, and in one copy in the cross-fertilized offspring of other individuals (Fisher 1941). Thus, if the fertilization success of self-fertilizing individuals is not compromised, and if the fitness of self-fertilized offspring is equal to the fitness of cross-fertilized offspring, selfing is expected to spread and go to fixation in a population.

The most important factor preventing the spread of self-fertilization is inbreeding depression (δ = 1 - fitness of self-fertilized offspring / fitness of cross-fertilized offspring), the reduced fitness of self-fertilized offspring in relation to cross-fertilized offspring. Early models for the evolution of self-fertilization found that if selfing does not affect outcrossing success, $\delta < \frac{1}{2}$ should favour the evolution of selfing while $\delta > \frac{1}{2}$ should favour the evolution of cross-fertilization (Lloyd 1979, Charlesworth 1980). This simple result has been used as a 'rule of thumb' in studies of mating system evolution, although it is known that several ecological (e.g., reproductive assurance) and genetic mechanisms (e.g., associations between loci determining mating system and fitness) may cause deviations from this prediction (see Charlesworth & Charlesworth 1987, Jarne & Charlesworth 1993, Uyenoyama et al. 1993, Holsinger 1996 for reviews).

Inbreeding depression is mostly due to (partially) recessive deleterious mutations that are expressed in homozygous state in the self-fertilized progeny, reducing their survival and reproduction (Charlesworth & Charlesworth 1999). Inbreeding depression is thus not a fixed parameter, but reflects the amount of segregating deleterious mutations in a population. The frequency of such mutations depends on several factors, the most important being the effective size of the population and the degree of inbreeding in the population. In small populations, and in populations practicing inbreeding, increased homozygosity exposes recessive deleterious alleles to selection and purging them from the population. Thus inbreeding depression is expected to decrease in populations that are inbred due either to small effective population size or to within-population inbreeding (Lande & Schemske 1985, Charlesworth et al. 1990, Wang et al. 1999, Bataillon & Kirkpatrick 2000, Hedrick 2002, Glémin 2003). Evidence that purging actually happens has been obtained from comparisons among species and populations differing in the amount of inbreeding, but it seems that purging is not always efficient (Husband & Schemske 1996, Byers & Waller 1999). It is known that slightly deleterious mutations with relatively strong heterozygous effects are not easily purged, and can cause substantial inbreeding depression also in inbred populations (Wang et al. 1999). Inbreeding depression may decrease in small populations also because in small populations, genetic drift reduces genetic variability. Without genetic variability there cannot be inbreeding depression.

The strength of inbreeding depression does not depend solely on the genetics of the population, but also on the environment where inbreeding depression is estimated. For example, it has often been observed that inbreeding depression may be stronger in harsh conditions (e.g., Dudash 1990, Jiminez et al. 1994, Keller et al. 2002) or when competition is more intense (Cheptou et al. 2000, Cheptou et al. 2001). One potentially important factor that may affect the strength of inbreeding depression is parasitism. Parasites are expected to disproportionately affect self-fertilized individuals because self-fertilized individuals have less genetic variability necessary to detect a wide selection of invading parasites. Also the poorer condition of individuals suffering from inbreeding depression may render them less able to fight off invading parasites. There is some empirical evidence, mostly from vertebrates, that inbred individuals and populations are more affected by parasites than

outbred individuals and populations (Mulvey et al. 1987, Coltman et al. 1999, Meagher 1999, Cassinello et al. 2001, Acevedo-Whitehouse et al. 2003), but other studies have found no such pattern (e.g., Trouvé et al. 2003). In experimental tests on the effect of inbreeding on parasite resistance, equivocal results have been obtained. Some studies have found no effect of inbreeding (Matheson et al. 1995, Salathè & Ebert 2003), while in some studies the effect is seen in some populations but not in others (Wiehn et al. 2002), or the effect depends on the species of parasite studied (Haag et al. 2003).

On top of inbreeding depression, the effects of the mating system on parental fecundity may also contribute to the relative fitness of self- and cross-fertilization. If self-fertilization results in lower fecundity than cross-fertilization, this will select against evolution of self-fertilization. The joint effect of mating system on parental fecundity and on offspring fitness has been termed self-fertilization depression (Jarne et al. 1991).

We studied the consequences of self- and cross-fertilization for viability, parental fecundity, hatching success of eggs and parasite resistance in eight natural populations of *Lymnaea stagnalis*. Our aim was to examine whether the natural mating systems of the snails could be understood in the light of the differential fitness consequences of self-and cross-fertilization.

1.3   Adverse effects of small population size

Understanding the extinction risks facing small populations is of central importance for the conservation of endangered species and populations. The issue is especially relevant in the present day because human land use continuously reduces and fragments natural habitats, causing declines in sizes and connectivity of natural populations. While demographic and environmental stochasticity are major threats to survival of small populations (Lande 1988), genetic changes taking place in small populations can additionally increase the risk of population extinction.

There are three main genetic threats facing small populations. Inbreeding depression — the unmasking of recessive, often highly detrimental alleles in the offspring of close relatives — can increase the probability of immediate extinction in populations suddenly reduced to small size (Newman & Pilson 1997, Saccheri et al. 1998). For populations that have declined in size more gradually, and for populations that have survived the initial first generations of close inbreeding, genetic load from fixation and accumulation of slightly deleterious mutations (i.e., drift load) may pose a more serious risk for population persistence. Slightly deleterious mutations can accumulate in small populations because genetic drift overrides natural selection against these mutations in small populations (Kimura et al. 1963, Wright 1977). In the long run, accumulation of mutations can reduce the reproductive output of individuals to the point that the population can no longer replace itself, resulting in extinction (e.g., Lande 1994, Lynch et al. 1995b).

The third genetic threat to small populations arises through a reduction in potentially adaptive genetic variation. In small populations, random genetic

drift causes stochastic fluctuations in allele frequencies, resulting in fixation or loss of alternative alleles at a locus. Low genetic variability may endanger the survival of populations since genetic variability is necessary for adaptation to changing environmental conditions. Often it is thought that the lack of adaptive genetic variability is not a serious threat to population survival because changes in the abiotic environment are usually very slow and variation in quantitative characters is restored quickly (Gaggiotti 2003). However, changes in the biotic environment, such as virulence and infectivity of parasitic diseases, may be very rapid, making the loss of adaptive variability an immediate threat to population survival (O'Brien & Evermann 1988, Allendorf & Ryman 2002, Altizer et al. 2003).

We were able to analyse the effects of small effective population size on key fitness parameters by correlating the amount of variability in the microsatellite loci with experimentally obtained fitness measures. Drift load in maturation age and fecundity was studied in a common laboratory environment, and the effect of heterozygosity on parasite resistance was studied in an experiment where snails were exposed to trematodes in a natural environment.

2   RESULTS AND DISCUSSION

2.1   Evolution of hermaphroditism versus separate sexes (I)

We built a mathematical model to study the consequences of mate search for the evolution of hermaphroditism versus separate sexes in animals. As all previous theories on hermaphroditism have to do with the advantages of hermaphroditism in situations where the availability of mating partners is limited, and as most animals are able to increase the number of encounters with potential mates by mate search, studying the effects of mate search to stability of hermaphroditism seemed warranted.

In the model, we examine how a trade-off between the number of potential mating partners and the amount of resources that can be allocated to reproduction affects the evolution of mating strategies. The model is based on two central trade-offs that any hermaphroditic animal is likely to face: a trade-off between time allocation to feeding and to mate search, and a trade-off between resource allocation to male and female sexual functions. Further, the two trade-offs are linked, since the amount of resources that can be allocated to sexual functions depends on the time the animal spends feeding. We analysed the model with analytical methods, using evolutionary game theory (Maynard Smith 1982) and with computer simulations that allowed us to study the joint evolution of time allocation and sex allocation.

Our model confirmed the previous results that hermaphroditism is favoured in populations with low population density, especially if the animals are not very efficient at mate search (i.e., there is a strong time allocation trade-off between feeding and mate search). In these conditions hermaphroditism has several advantages: first, the possibility of reproducing by self-fertilization may favour hermaphroditism even if inbreeding depression is very high; second, cross-fertilization is facilitated by the fact that all encountered individuals are suitable mating partners; and third, because matings are few, only a small fraction of resources are allocated to male function. Low allocation to male function favours hermaphroditism over separate sexes because in such

populations the average fitness of hermaphrodites is higher than the fitness of pure males and females.

Under natural conditions, population densities of hermaphroditic animals are often so high that cross-fertilization is not limited by the availability of mates (Michiels 1999). We found that under such conditions mate search efficiency determines whether the population remains hermaphroditic or whether separate males and females evolve. If mate search is inefficient, that is, if an animal cannot drastically increase the number of encounters by searching for mates, or if the costs of mate search are high, hermaphroditism is stable. Further, because resource allocation is female biased in hermaphroditic populations, such populations have very high reproductive output. Perhaps more interesting is what happens in populations with efficient mate search. Here, the population diverges to searching and non-searching phenotypes, and ultimately to males and females. The reason for this divergence is that when encounters and matings become more frequent in the population, the profitability of mating in the male role decreases. In this situation, more can be gained from refraining from searching for partners and instead specializing on production of female gametes. However, not every individual should become a female. Actually, because the average fitness of male and female functions is by necessity the same (Fisher 1930), half of the population specializes in the male function and half in the female function.

Depending on how willing individuals are to mate in the female role, slightly different results are obtained for high-density populations. In general, the more ready individuals are to mate in the female role, the more stable hermaphroditism is. If individuals restrict the number of matings as female, evolution of separate sexes becomes more likely.

Our results show that hermaphroditism is intrinsically unstable in species with good powers of locomotion and sensory capabilities for locating partners. This finding may explain the rarity of simultaneous hermaphroditism in so-called higher animals. Where hermaphroditism occurs in such animals, for example some fish species (Fischer & Petersen 1987, Petersen 1991), it is invariably associated with special mating behaviours that limit the potential for specialization in male role. The fact that species with good abilities to locate partners are hermaphroditic only when there is some special mating system to limit reproductive success through the male function may be considered as supportive evidence for our model.

As with any model, our results are valid as far as the assumptions of the model are correct. We have not considered all the possible factors that may influence the evolution of separate sexes versus hermaphroditism, of which behavioural mating tactics may well be important. For example, we have not studied how mate choice and mate discrimination will affect the results, although it is likely that including mate discrimination in the model would make the evolution of separate sexes more likely. On the other hand, if the animals mate reciprocally, i.e., they donate sperm only if they also receive sperm, hermaphroditism will be stable because the evolution of pure females is prevented. Further theoretical work could be done to study the effects of mating tactics on gender evolution.

2.2   Mating system evolution in *Lymnaea stagnalis* populations

We studied the evolution of self- and cross-fertilization in eight populations of the great pond snail *Lymnaea stagnalis*. This snail is a self-compatible hermaphrodite (e.g., Cain 1956), but the natural mating system of the species has not been studied before. We inferred the natural mating system in our study populations using microsatellite genetic markers (II). The genetic markers were also used to study the degree of isolation among populations, and the size of the populations in the past.

We adopted both comparative and experimental approaches to the study of factors that may be directing the evolution of mating systems in our study populations. In the natural populations we quantified the density of snails, as low density may favour the evolution of self-fertilization. We also estimated the prevalence of trematode parasites infecting snails in the natural populations because strong parasite pressure may favour evolution of cross-fertilization as a means of escaping co-evolving parasites. In laboratory, we quantified the consequences of self- vs. cross-fertilization to parental fecundity and to offspring survival. Further, we experimentally studied the effect of the mating system on the resistance to trematode parasites. All manuscripts deal with a different set of questions, and the key data is original to each manuscript. However, some of the data has been used in more than one manuscript to gain a comprehensive treatment of the specific study questions. The cases where same data has been used in more than one manuscript are laid out in Table 1.

TABLE 1   Data reported in more than one manuscript of the thesis.

Data	In manuscripts
Inbreeding coefficient (F_{IS}) (cohorts combined)	III, IV, V
Allelic richness (A)	III, V
Expected heterozygosity (H_e)	IV, V
Snail density in natural populations	III, V
Snail fecundity in laboratory	III, V (different statistical models and sub-samples of snails)

2.2.1 Genetic analysis of the study populations (II, III, V)

Adult snails, and snails originating from field-collected eggs, were genotyped for nine microsatellite loci. Genotypic data revealed that some self-fertilization was taking place in all populations. However, in every population, cross-fertilization was the predominant mode of reproduction. Mating systems of all

populations were remarkably similar, despite the strong genetic isolation observed between populations.

Separate analysis of adult and juvenile cohorts indicated that self-fertilized snails did not suffer increased mortality compared to outcrossed snails, that is, there is no significant inbreeding depression in viability in nature. This analysis however assumes that the mating system does not change between generations, and we cannot directly test whether this assumption is valid. Nevertheless, the modest among-population variation in the mating system suggests that the within-population variation may also be low.

The amount of genetic variability, measured as allelic richness (a measure of allele number independent of sample size (Petit et al. 1998)) and expected heterozygosity varied considerably among populations. The amount of genetic variability can be used as an indicator of past effective population sizes: the less genetic variability, the smaller the population has been (Nei et al. 1975). Historical population sizes are relevant to mating system evolution because population size may affect the magnitude of inbreeding depression: small population size will purge highly recessive deleterious mutations, and genetic drift will reduce the amount of genetic variability (Wang et al. 1999, Glémin 2003).

2.2.2 Ecological correlates of mating system (III, V)

We investigated whether ecological variables, namely snail density and prevalence of trematode parasites, could explain some of the among-population variation in the amount of self-fertilization.

Density of snails in the populations varied considerably. However, all populations were mainly cross-fertilizing, and there was no correlation between snail density and the mating system. This result suggests that low density of snails is not limiting cross-fertilization in *Lymnaea stagnalis* populations. It is known that these snails can store and utilize sperm received from another individual for about one hundred days (Cain 1956). The ability to store sperm may allow for cross-fertilization in conditions where encounters between mating partners are infrequent, as would seem to be the case in our populations with low snail densities.

The prevalence of trematode parasites infecting the snails also varied considerably among populations. The prevalence of parasite infection increased during summer, and reached very high levels in two of the populations by mid July, when the last estimate of parasite prevalence was done. The correlation between mating system and parasite prevalence was in the direction predicted by theory: self-fertilization was more common in populations with lower parasite prevalence (Pearson $r = -0.543$, P = 0.164 for the last sampling date). However, as the mating systems did not vary appreciably between populations, and as the correlation was not statistically significant, no conclusion about the importance of parasites in determining the mating system of our study populations can be drawn.

2.2.3 Effects of mating system on fecundity and hatching success (III)

We studied the consequences of self- vs. cross-fertilization for parental fecundity and for offspring survival in a controlled laboratory experiment. Snails collected from the study populations as eggs were grown to maturity in isolation in laboratory. Grown snails were divided to two treatments. Snails in the self-fertilization treatment were forced to reproduce by self-fertilization. Snails in the cross-fertilization treatment we paired with another snail, and the behaviour of the snail pair was monitored until the snail designated to the cross-fertilization treatment had mated in the female role. After the mating system treatment the snails were isolated, their fecundity was recorded, and a subset of eggs was taken for estimation of hatching success.

Fecundity of snails did not differ between the mating system treatments, demonstrating that the evolution of self-fertilization in the study populations is not limited by the ability of snails to produce self-fertilized eggs.

Hatching success of eggs was affected by the mating system treatment: on the experiment-wide level self-fertilized eggs were less likely to hatch than cross-fertilized eggs. On the population level, inbreeding depression in hatching success was statistically significant in three of the study populations, but non-significant in five populations. In general, inbreeding depression was low to moderate. As all our study populations showed evidence of partial selfing and small effective population size, low inbreeding depression in hatching success was expected. Mutations affecting early viability are thought to be highly recessive and thus easily purged by partial selfing or inbreeding due to small population size.

2.2.4 Effects of the mating system on resistance to trematode parasites (IV)

We experimentally studied the effect of mating system on resistance to trematode parasites. Trematode parasites are very common in our populations, and they have a strong impact on the fitness of the snails because they castrate the snails they infect. If self-fertilized snails are more susceptible to these parasites than cross-fertilized snails, trematodes could act as a selective force favouring the evolution of cross-fertilization.

The experimental snails were the self- and cross-fertilized offspring of snails collected from the study populations as eggs. Mating system of the parental snails (self- or cross-fertilization) was manipulated as described in the previous section. From each population by treatment combination we chose one egg mass from nine to twelve snails; snails hatching from these eggs became the experimental animals in the study.

The experimental snails were grown in the laboratory over winter and transplanted to Lake Jyväsjärvi on June 20, 2000. In the lake, the snails were housed in cylinder shaped plastic cages where they were exposed to naturally occurring trematode larva. The cages were organized in a row parallel to the shore. From each population by treatment combination, snails were allocated to three to five randomly chosen cages. Snails were fed with lettuce three times a week. After nine weeks, the snails were taken from the lake and screened for trematode infection.

The mating system did not have an effect on the probability of infection: self- and cross-fertilized snails were equally likely to be infected by trematode parasites. Thus it seems that parasites are not likely to contribute to inbreeding depression and to mating system evolution in our study populations. Interestingly however, when we pooled the data from all populations, we found microsatellite heterozygosity to be significantly associated with parasite infection: more heterozygous snails were less likely to be infected. This result will be discussed further in the next section. We also found an effect of the spatial position of the cage to trematode prevalence. The spatial effect most likely reflects a cline in the density of the infective trematode larvae along the shore. Such a cline arises easily if an infected final host (a bird or a mammal) preferentially habits a certain location on the shore. Spatial covariation between final host density and proportion of infected snails has been observed in numerous studies (e.g., Jokela & Lively 1995, Smith 2001).

2.3 Consequences of small population size

Population genetic analysis revealed that our study populations are highly isolated, and the amount of genetic variability varies considerably between populations. Some of the populations had lost almost all variation in the examined microsatellite loci, suggesting that these populations have either gone through very small population bottleneck(s), or have been isolated from other populations for a very long time. Other populations harboured more genetic variation, suggesting that these populations have retained larger population sizes.

Population genetic theory suggests that in small populations, slightly deleterious mutations may accumulate and decrease the fitness of organisms (e.g., Kimura et al. 1963, Lande 1994, Lynch et al. 1995a). Thus populations with lower variability at neutral loci (smaller historical population size) are predicted to have lower reproductive output than populations with higher variability at neutral loci (larger historical population size). Small effective population size also decreases the amount of potentially adaptive genetic variability that may be especially important for the ability to detect and defend against parasites and diseases.

2.3.1 Drift load in reproductive output (V)

We studied the relationship between genetic variability and reproductive output in the eight *Lymnaea stagnalis* populations. Snails collected from the populations as eggs were brought to laboratory and their maturation age and fecundity were estimated. To relate the results to the natural populations, we also analysed the density of snails and the prevalence of trematode infections in the original populations.

Both maturation age and fecundity were strongly and significantly correlated with the amount of genetic variability in populations. Low genetic variability was associated with slow maturation and low fecundity. Differences

in maturation age and fecundity among populations were large. In natural conditions, snails in the poorest populations probably mature about one year later than snails in the genetically more variable populations. Differences in fecundity were correspondingly high: the best population had five-fold fecundity compared to the poorest population. An important point in the analysis was that we were able to show that the relationship between genetic variability and reproductive output was independent of the amount of non-random mating in the populations. Thus our results were not confounded by possible inbreeding depression following self-fertilization in the populations.

Perhaps surprisingly, densities of snails in the natural populations were not correlated with the amount of genetic variability. Thus the current population densities do not reflect the historical population sizes of the snails. Instead we found that prevalence of trematode infection was negatively correlated with snail density. As the prevalence of trematode infection was very high in some of the populations, and infection results in castration of the snail, it seems likely that trematode parasites may determine, at least in part, the densities of snails in the populations. Other studies have shown that the prevalence of trematode infection in snails is largely determined by the presence and movements of definitive hosts (Sousa 1990, Jokela and Lively 1995, Esch et al. 2001, Smith 2001). As the most important definitive hosts in our populations are migratory waterfowl, the exposure of snails to trematodes can potentially show great temporal variation. Perhaps variation in the prevalence of trematode infection causes fluctuations in population size and increases the amount of genetic drift in our study populations.

These results give strong empirical support to theoretical models predicting decreased fitness in small and isolated populations. Gradual erosion of fitness by genetic drift is especially dangerous because it may go unnoticed in populations regulated by ecological factors until the reproductive rate is so low that extinction is unavoidable.

2.3.2 Heterozygosity and parasite resistance (IV)

We estimated the relationship between heterozygosity at the microsatellite loci and the probability of trematode infection from the study described in section 2.2.4. We assume that variation at the microsatellite loci reflects the amount of variation at loci determining trematode resistance. As practically nothing is known about the specific genes controlling trematode resistance in snails, neutral markers are the best surrogate for studying the association between genetic variability and disease resistance.

Although the mating system treatment did not have a significant effect on trematode resistance, as described in section 2.2.4, we found that there was an overall negative correlation between snail heterozygosity and the probability of trematode infection. The negative relationship was detected both at population level, and at the level of population by treatment combinations. These results suggest that heterozygosity is an important determinant of resistance to trematode infection, and that parasitic diseases may cause an elevated risk of extinction in populations lacking genetic variability.

3 CONCLUSIONS

This thesis is focused on three different topics: evolution of hermaphroditism versus separate sexes in animals, evolution of self- versus cross-fertilization in the great pond snail *Lymnaea stagnalis*, and the consequences of small historical population size to reproductive output and to parasite resistance in *L. stagnalis* populations.

In the theoretical part of the thesis we explored the evolution of hermaphroditism versus separate sexes in animals. The study revealed that the ability of animals to locate possible mating partners could determine whether hermaphroditism is evolutionary stable, or whether separate males and females evolve. When mate-search is inefficient, due to low density or low mobility of animals, or to high costs of searching, hermaphroditism is evolutionarily stable. However, if mate search is efficient, disruptive selection on time allocation between searching and feeding leads to evolution of searching and non-searching individuals, and ultimately to evolution of males and females. This result may explain why animals with good powers of locomotion typically have males and females, and why sluggish animals often are hermaphroditic.

The study on the evolution of self- versus cross-fertilization in *Lymnaea stagnalis* populations revealed that cross-fertilization is the predominant mode of reproduction in all our study populations. Cross-fertilization prevailed even in low-density populations, possibly because these snails are able to store sperm for long periods of time. The high degree of outcrossing is surprising, given that we found only limited inbreeding depression for hatching success of eggs, and no parental fecundity disadvantage of self-fertilization. Further, population genetic analysis of two cohorts of snails did not show evidence for inbreeding depression in viability in natural conditions, and mating system did not have an effect on probability of infection by trematode parasites. Together, these results suggest that inbreeding depression may be quite low in the study populations. However, it may be that we have underestimated inbreeding depression because we did not study the effects of mating system on offspring fecundity, where inbreeding depression is commonly observed even if early life-history traits show no inbreeding depression.

The study on the effects of past population size to reproductive output revealed a very strong negative effect of small historical population size to snail maturation age and to fecundity. Current densities of snails in the populations were however not related to the estimates of reproductive output. Instead, there was suggestive evidence that snail densities were partly determined by the prevalence of trematode infection in the populations. These results are highly relevant to conservation of endangered species and populations, as they give strong empirical support to theoretical models predicting decreased fitness in small and isolated populations. This type of fitness deterioration may be especially dangerous because it may go unnoticed in populations regulated by ecological factors until the reproductive rate is so low that extinction is unavoidable.

In the study focusing on the effects of mating system and genetic variability on resistance to trematode parasites, a similar result was found: genetically less diverse snails were more likely to become infected. Loss of genetic variability may thus increase the extinction risk of populations with diminished genetic variability.

Acknowledgements

Many people contributed to this thesis in many different ways. First I thank Veijo Kaitala, my supervisor, for continued support to the project that has taken a good bit longer than I imagined at the beginning. Warm-hearted thanks to Emily Knott; your help to this work has been invaluable. Kari Nissinen generously offered statistical advice, and also performed many analyses. Warm thanks are also due to the master students Susanna Suonpää, Tapio van Ooik and Mirjami Hytönen who did much of the hands on work and also took the responsibility for smooth operation of many experiments. Jouni Taskinen introduced me to the snails and their parasites, without him I might still be working *in silico*. Warm thanks are also due to the trainees that have been involved in doing the experiments and in the DNA laboratory: Nina Göös, Tarmo Ketola, Tuuli Kontio, Jani Kupari, Salla Ruskamo and Anni Pursiainen.

I am not able to thank all the numerous people that have influenced this thesis work by name. I will anyhow acknowledge Rauno Alatalo who has been heading the ecology section during most of my studies in Jyväskylä, and has made it the dynamic and productive unit it is. Katri Kärkkäinen and Silja Parri helped in starting up the molecular work with the snails. Department staff never spared their efforts in organizing practical matters, and I'd like to especially thank Juha Ahonen and Tarmo Halinen for the support given to my studies.

Huge thanks also to everybody at the department: faculty, researchers, and fellow students. I have enjoyed working with you all, and I consider you more as friends than colleagues. I refrain from making any name list because no matter how long, it would still be incomplete. Thanks also to friends in the APS for keeping in touch and for memorable outings around southern and central Finland.

Finally I wish to thank my family and Saija's family for support and for enjoyable holidays in Espoo and in Rovaniemi. And the greatest thanks go to Saija for her love and for her toleration of my absent-mindedness and long working days, and to Tuuli for making every day very special.

22

YHTEENVETO

Kaksineuvoisuus eli yksilön kyky tuottaa sekä naaras- että koirassukusoluja on hyvin yleistä kasveilla, mutta sitä esiintyy laajalti myös eläinkunnassa, muun muassa laaka- ja harvasukamadoilla sekä kotiloilla. Luonnonvalinta voi suosia kaksineuvoisuutta, koska kaksineuvoiset eliöt voivat allokoida resursseja koiras- ja naarastoimintoihin tilanteen mukaan optimoiden, ja täten parantaa lisääntymismenestystään. Luonnonvalinta voi suosia kaksineuvoisuutta myös tilanteissa, joissa parittelukumppanin löytäminen on vaikeaa, sillä kaksineuvoisessa populaatiossa jokainen lajitoveri on sopiva parittelukumppani.

Tässä väitöskirjassa olen matemaattisen mallinnuksen välinein tutkinut tekijöitä, jotka suosivat kaksineuvoisuuden, taikka erillisten koiras- ja naarasyksilöiden, evoluutiota eläinpopulaatioissa. Luonnonvalinnan uskotaan suosivan kaksineuvoisuutta erityisesti tilanteissa, joissa parittelukumppanin löytäminen on vaikeaa esimerkiksi alhaisen yksilötiheyden vuoksi. Useimmat eläimet pystyvät kuitenkin aktiivisella etsinnällä lisäämään kohtaamiensa mahdollisten parittelukumppanien määrää. Kumppaninetsinnän vaikutuksia kaksineuvoisuuden evoluutioon ei kuitenkaan ole aiemmin tutkittu. Tutkimuksessamme selvisi, että kumppaninetsinnän tehokkuus ratkaisee sen, suosiiko luonnonvalinta kaksineuvoisuutta vaiko erillisten koiraiden ja naaraiden evoluutiota. Jos etsintä on tehotonta tai etsinnästä koituvat kustannukset suuret (kuten esim. kotiloilla, joilla liikkuminen vaatii suuria määriä energiaa), on kaksineuvoisuus evolutiivisesti vakaa lisääntymistapa. Tehokas kumppaninetsintä taas johtaa tilanteeseen, jossa luonnonvalinta suosii kahta erilaista strategiaa: toinen strategia on etsiä mahdollisimman monia kumppaneita, joiden kanssa paritella ja toinen on pidättäytyä etsinnästä ja erikoistua naarassukusolujen tuotantoon. Tämä hajottava valinta johtaa lopulta koiraiden ja naaraiden evoluutioon populaatioissa, joissa yksilöt kykenevät paikallistamaan ja saavuttamaan lajikumppaneitaan suhteellisen vaivattomasti.

Useimmat kaksineuvoiset eliöt kykenevät lisääntymään myös ilman kumppania itsesiitoksella eli hedelmöittämällä naarassukusolunsa omilla koirassukusoluillaan. Teoriassa itsesiitoksen tulisi yleistyä kaksineuvoisten eliöiden populaatiossa, sillä itsesiittoisuutta aiheuttavan geenin leviämistehokkuus populaatiossa on ristisiittoisuutta aiheuttavaa geeniä suurempi. Itsesiittoinen yksilö saa seuraavaan sukupolveen kaksi kopiota geeneistään jokaisessa itsesiitetyssä jälkeläisessä ja yhden jokaisessa siittämässään toisen yksilön tuottamassa jälkeläisessä, kun taas ristisiittoisen yksilön geeneistä siirtyy vain yksi kopio sekä itse tuotettuihin että toisten yksilöiden tuottamiin jälkeläisiin. Ristisiitos voi kuitenkin säilyä populaatiossa, jos itsesiittoiset jälkeläiset kärsivät sisäsiitosheikkoudesta eli alentuneesta elinkyvystä ja lisääntymistehosta verrattuna ristisiittoisiin jälkeläisiin. Mikäli itsesiitettyjen jälkeläisten kelpoisuus on alle puolet ristisiitettyjen jälkeläisten kelpoisuudesta, ei itsesiitos voi yleistyä populaatiossa.

Sisäsiitosheikkous johtuu pääasiassa haitallisista väistyvistä mutaatioista, jotka ilmenevät homotsygootteina itsesiitetyissä jälkeläisissä ja heikentävät niiden kelpoisuutta. Haitallisten väistyvien mutaatioiden määrä, ja siten myös sisäsiitosheikkouden voimakkuus, riippuu muun muassa populaation sisäsii-

tosasteesta. Sisäsiittoisissa populaatioissa haitallisten väistyvien mutaatioiden määrä vähenee, koska sellaisten yksilöiden, joilla on useita tällaisia mutaatiota homotsygootteina, kelpoisuus on alhainen, eivätkä mutaatiot siten siirry seuraavaan sukupolveen. Kun toisaalta itsesiitoksen yleistyminen riippuu sisäsiitosheikkouden asteesta (alhainen sisäsiitosheikkous suosii itsesiitoksen yleistymistä), ovat itsesiitoksen määrä ja sisäsiitosheikkous vuorovaikutteisessa suhteessa toisiinsa. Tästä vuorovaikutteisesta suhteesta johdettu teoria ennustaa, että luonnossa sisäsiittoisilla populaatiolla tulisi olla vain vähän sisäsiitosheikkoutta ja ristisiittoisilla populaatiolla korkea sisäsiitosheikkous.

Populaation sisäsiitosaste voi nousta kahdella tapaa: kun lähisukuiset yksilöt pariutuvat keskenään useammin kuin sattumanvaraisen pariutumisen perusteella voisi olettaa (itsesiitos on äärimmäistä sisäsiitosta, sillä yksilö pariutuu itsensä kanssa) tai kun populaatio on niin pieni, että sukulaisuusaste yksilöiden välillä vääjäämättä nousee korkeaksi. Pieni populaatiokoko voi siis nostaa populaation sisäsiitosastetta ja siten poistaa populaatiosta haitallisia väistyviä mutaatiota sekä vähentää sisäsiitosheikkoutta. Mutta pienestä populaatiokoosta voi olla myös haitallisia seurauksia populaation elinkyvylle, sillä pienessä populaatiossa satunnainen geneettinen ajautuminen mahdollistaa lievästi haitallisten mutaatioiden yleistymisen. Ajan mittaan tällaiset lievästi haitalliset mutaatiot voivat keräytyä populaatioon ja vähentää yksilöiden lisääntymistehoa niin paljon, että populaatio ei enää kykene uusiutumaan vaan ajautuu sukupuuttoon.

Pieni populaatiokoko johtaa myös geneettisen vaihtelun määrän vähenemiseen, koska harvinaisemmat alleelit katoavat populaatiosta geneettisen ajautumisen seurauksena. Populaation aiempi koko voidaankin selvittää tutkimalla ns. neutraalin geneettisen vaihtelun määrää populaatiossa: mitä vähemmän vaihtelua, sitä pienempi (ja/tai eristyneempi) populaatio on ollut. Pieni populaatiokoko hävittää myös yksilöiden fenotyyppiin vaikuttavaa geneettistä vaihtelua. Fenotyyppiin vaikuttavan geneettisen vaihtelun häviäminen voi olla vaaraksi populaation säilymiselle, sillä se on tarpeen muuttuviin ympäristöolosuhteisiin sopeuduttaessa. Erityisen haitallista voi olla vaihtelun häviäminen niissä geeneissä, jotka vaikuttavat puolustukseen tauteja ja loisia vastaan.

Väitöskirjassani tutkin empiirisesti itse- ja ristisiitoksen evoluutiota isolimakotilo (*Lymnaea stagnalis*) -populaatiossa sekä populaatiokoon vaikutusta kotiloiden lisääntymistehoon ja kykyyn vastustaa imumatoloisia. Analysoimalla kotiloiden perimän neutraalia geneettistä vaihtelua (ns. mikrosatelliitti-DNA:ta) saimme selville, että kaikissa kahdeksassa tutkimuspopulaatiossa vallitseva lisääntymistapa oli ristisiitos. Kokeellisissa tutkimuksissa selvisi, että itsesiitoksesta ei koitunut kovin suuria kustannuksia vanhemman jälkeläistuottoon eikä jälkeläisten elinkykyyn. Itsesiitetyt kotilot eivät myöskään olleet alttiimpia loisinnalle kuin ristisiitetyt kotilot. Nämä tulokset viittaavat siihen, että sisäsiitosheikkous kotilopopulaatiossa on varsin alhainen, mutta lopullista arviota sisäsiitosheikkouden määrästä ei voi tutkimustemme perusteella antaa. Mikäli sisäsiitosheikkous kuitenkin on alhainen, kuten tutkimuksemme antavat olettaa, on populaatioiden korkea ristisiitosaste hieman yllättävä, sillä tällaisissa populaatioissa itsesiitoksen pitäisi teoriassa yleistyä nopeasti.

Populaatiogeneettiset analyysit paljastivat myös, että tutkimuspopulaatioidemme koot ovat aiemmin olleet hyvin erilaiset. Osa populaatioista on ollut

hyvin pieniä (ja/tai eristäytyneitä), ja näistä populaatioista oli suuri osa geneettistä vaihtelua hävinnyt. Toiset populaatiot taas ovat olleet suurempia; näissä populaatioissa geneettinen vaihtelu oli runsaampaa. Nykyinen ja geneettisestä aineistosta päätelty aiempi populaatiokoko eivät vastanneet toisiaan. Maastosta kerätty aineisto viittasi siihen, että nykyiset populaatiokoot selittyvät ainakin osin imumatoloisinfektioiden yleisyydellä: mitä enemmän loisia, sitä pienempi populaatiokoko.

Kokeellisissa tutkimuksissa havaittiin, että aiempi pieni populaatiokoko oli yhteydessä kotiloiden myöhentyneeseen aikuistumiseen ja matalaan jälkeläistuottoon, jotka yhdessä huomattavasti alensivat kotiloiden lisääntymistehoa. Alentunut lisääntymisteho on todennäköisesti seurausta lievästi haitallisten mutaatioiden kertymisestä pieniin populaatioihin. Lisääntymistehon aleneminen pienissä populaatiossa voi vaarantaa populaatioiden pitkän aikavälin säilymisen, ja tämä tulisi huomioida uhanalaisten lajien ja populaatioiden suojelua suunniteltaessa.

Kokeellisissa tutkimuksissa havaittiin myös, että kotilon heterotsygotiaaste, eli yksilön sisäisen geneettisen vaihtelun määrä, vaikutti vahvasti kotilon riskiin saada imumatoloisinfektio. Tämä tulos viittaa siihen, että geneettisen vaihtelun väheneminen voi olla myös välitön riski populaatioiden säilymiselle.

REFERENCES

Acevedo-Whitehouse, K., Gulland, F., Greig, D. & Amos, W. 2003. Disease susceptibility in California sea lions. Nature 422, 35.

Allendorf, F. W. & Ryman, N. 2002. The role of genetics in population viability analysis. In: S. R. Beissinger & D. R. McCullough (eds), Population viability analysis. Chicago: University of Chicago Press.

Altenburg, E. 1934. A theory of hermaphroditism. American Naturalist 68, 88-91.

Altizer, S., Harvell, D. & Friedle, E. 2003. Rapid evolutionary dynamics and disease threats to biodiversity. Trends in Ecology & Evolution 18, 589-596.

Bataillon, T. & Kirkpatrick, M. 2000. Inbreeding depression due to mildly deleterious mutations in finite populations: size does matter. Genetical Research 75, 75-81.

Byers, D. L. & Waller, D. M. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. Annual Review of Ecology and Systematics 30, 479-513.

Cain, G. L. 1956. Studies on cross-fertilization and self-fertilization in Lymnaea stagnalis appressa Say. Biological Bulletin (Woods Hole) 111, 45-52.

Cassinello, J., Gomendio, M. & Roldan, E. R. S. 2001. Relationship between coefficient of inbreeding and parasite burden in endangered gazelles. Conservation Biology 15, 1171-1174.

Charlesworth, B. 1980. The cost of sex in relation to mating system. Journal of Theoretical Biology 84, 655-671.

Charlesworth, B. & Charlesworth, D. 1999. The genetic basis of inbreeding depression. Genetical Research 74, 329-340.

Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18, 237-268.

Charlesworth, D., Morgan, M. T. & Charlesworth, D. 1990. Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage. Evolution 44, 1469-1489.

Charnov, E. L., Maynard Smith, J. & Bull, J. J. 1976. Why be an hermaphrodite? Nature 263, 125-126.

Cheptou, P.-O., Imbert, E., Lepart, J. & Escarre, J. 2000. Effects of competition on lifetime estimates of inbreeding depression in the outcrossing plant Crepis sancta (Asteraceae). Journal of Evolutionary Biology 13, 522-531.

Cheptou, P.-O., Lepart, J. & Escarre, J. 2001. Inbreeding depression under intraspecific competition in a highly outcrossing population of Crepis sancta (Asteraceae): evidence for frequency dependent variation. American Journal of Botany 88, 1424-1429.

Coltman, D. W., Pilkington, J. G., Smith, J. A. & Pemberton, J. M. 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. Evolution 53, 1259-1267.

Darwin, C. 1876. The effects of cross and self fertilisation in the vegetable kingdom. London: J. Murray.

Doums, C., Viard, F. & Jarne, P. 1998. The evolution of phally polymorphism. Biological Journal of the Linnean Society 64, 273-296.

Dudash, M. R. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, Sabatia angularis L. (Gentianaceae): a comparison in three environments. Evolution 44, 1129-1139.

Esch, G. W., Curtis, L. A. & Barger, M. A. 2001. A perspective on the ecology of trematode communities in snails. Parasitology 123, S57-S75.

Fischer, E. A. & Petersen, C. W. 1987. The evolution of sexual patterns in the seabasses. Bioscience 37, 482-489.

Fisher, R. A. 1930. The Genetical Theory of Natural Selection. Oxford: Clarendon Press.

Fisher, R. A. 1941. Average excess and average effect of a gene substitution. Annals of eugenetics 11, 53-63.

Gaggiotti, O. E. 2003. Genetic threats to population persistence. Annales Zoologici Fennici 40, 155-168.

Ghiselin, M. T. 1969. The evolution of hermaphroditism among animals. The Quarterly Review of Biology 44, 189-208.

Ghiselin, M. T. 1974. The Economy of Nature and the Evolution of Sex. Berkeley, CA: University of California Press.

Glémin, S. 2003. How are deleterious mutations purged? Drift versus nonrandom mating. Evolution 57, 2678-2687.

Haag, C. R., Sakwinska, O. & Ebert, D. 2003. Test of synergistic interaction between infection and inbreeding in Daphnia magna. Evolution 57, 777-783.

Heath, D. J. 1977. Simultaneous hermaphroditism: cost and benefit. Journal of Theoretical Biology 64, 363-373.

Hedrick, P. W. 2002. Lethals in finite populations. Evolution 56, 654-657.

Holsinger, K. E. 1996. Pollination biology and the evolution of mating systems in flowering plants. Evolutionary Biology 29, 107-149.

Husband, B. C. & Schemske, D. W. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50, 54-70.

Jarne, P. & Charlesworth, D. 1993. The evolution of the selfing rate in functionally hermaphrodite plants and animals. Annual Review of Ecology and Systematics 24, 441-466.

Jarne, P., Finot, L., Delay, B. & Thaler, R. 1991. Self-fertilization versus cross-fertilization in the hermaphroditic freshwater snail Bulinus globosus. Evolution 45, 1136-1146.

Jiminez, J. A., Hughes, K. A., Alaks, G., Graham, L. & Lacy, R. C. 1994. An experimental study of inbreeding depression in a natural habitat. Science 266, 271-273.

Jokela, J. & Lively, C. M. 1995. Spatial variation in infection by digenetic trematodes in a population of freshwater snails (Potamopyrgus antipodarum). Oecologia 103, 509-517.

Keller, L. F., Grant, P. R., Grant, B. R. & Petren, K. 2002. Environmental conditions affect the magnitude of inbreeding depression in survival of Darwin's finches. Evolution 56, 1229-1239.

Kimura, M., Maruyama, T. & Crow, J. F. 1963. The mutation load in small populations. Genetics 48, 1303-1312.

Lande, R. 1988. Genetics and demography in biological conservation. Science 241, 1455-1460.

Lande, R. 1994. Risk of population extinction from fixation of new deleterious mutations. Evolution 48, 1460-1469.

Lande, R. & Schemske, D. W. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution 39, 24-40.

Lloyd, D. G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. American Naturalist 113, 67-79.

Lynch, M., Conery, J. & Bürger, R. 1995a. Mutation accumulation and the extinction of small populations. American Naturalist 146, 489-518.

Lynch, M., Conery, J. & Bürger, R. 1995b. Mutational meltdowns in sexual populations. Evolution 49, 1067-1080.

Matheson, A. C., White, T. M. & Powell, G. R. 1995. Effects of inbreeding on growth, stem form and rust resistance in Pinus elliottii. Silvae Genetica 44, 37-43.

Maynard Smith, J. 1978. The Evolution of Sex. Cambridge: Cambridge University Press.

Maynard Smith, J. 1982. Evolution and the Theory of Games. Cambridge: Cambridge University Press.

Meagher, S. 1999. Genetic diversity and Capillaria hepatica (Nematoda) prevalence in Michigan deer mouse populations. Evolution 53, 1318-1324.

Michiels, N. K. 1999. Sexual adaptations to high density in hermaphrodites. Invertebrate Reproduction and Development 36, 35-40.

Mulvey, M., Goater, T. M., Esch, G. W. & Crews, A. E. 1987. Genotype frequency differences in Halipegus occidualis-infected and uninfected Helisoma anceps. Journal of Parasitology 73, 757-761.

Nei, M., Maruyama, T. & Chakraborty, R. 1975. The bottleneck effect and genetic variability of populations. Evolution 29, 1-10.

Newman, D. & Pilson, D. 1997. Increased probability of extinction due to decreased genetic effective population size: experimental populations of Clarkia pulchella. Evolution 51, 354-362.

O'Brien, S. J. & Evermann, J. F. 1988. Interactive influences of infectious disease and genetic diversity in natural populations. Trends in Ecology & Evolution 10, 254-259.

Petersen, C. W. 1991. Sex allocation in hermaphroditic sea basses. American Naturalist 138, 650-667.

Petit, R. J., El Mosaudik, A. & Pons, O. 1998. Identifying populations for conservation on the basis of genetic markers. Conservation Biology 12, 844-855.

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P. & Hanski, I. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392, 491-494.

Salathè, P. & Ebert, D. 2003. The effects of parasitism and inbreeding on the competitive ability in Daphnia magna: evidence for synergistic epistasis. Journal of Evolutionary Biology 16, 976-985.

Smith, N. F. 2001. Spatial heterogeneity in recruitment of larval trematodes to snail intermediate hosts. Oecologia 127, 115-122.

Sousa, W. P. 1990. Spatial scale and the processes structuring a guild of larval trematode parasites. In: G. W. Esch, A. O. Bush & J. M. Aho (eds), Parasite communities: patterns and processes. London: Chapman and Hall.

Tomlinson, J. 1966. The advantages of hermaphroditism and parthenogenesis. Journal of Theoretical Biology 11, 54-58.

Trouvé, S., Degen, L., Renaud, F. & Goudet, J. 2003. Evolutionary implications of a high selfing rate in the freshwater snail Lymnaea truncatula. Evolution 57, 2303-2314.

Uyenoyama, M. K., Holsinger, K. E. & Waller, D. M. 1993. Ecological and genetic factors directing the evolution of self-fertilization. Oxford Surveys in Evolutionary Biology 9, 327-381.

Wang, J., Hill, W. G., Charlesworth, D. & Charlesworth, B. 1999. Dynamics of inbreeding depression due to deleterious mutations in small populations: mutation parameters and inbreeding rate. Genetical Research 74, 165-178.

Wiehn, J., Kopp, K., Rezzonico, S., Karttunen, S. & Jokela, J. 2002. Family-level covariation between parasite resistance and mating system in a hermaphroditic freshwater snail. Evolution 56, 1454-1461.

Wright, S. 1977. Evolution and the genetics of populations. Chicago: University of Chicago Press.

ORIGINAL PAPERS

I

MATE-SEARCH EFFICIENCY CAN DETERMINE THE EVOLUTION OF SEPARATE SEXES AND THE STABILITY OF HERMAPHRODITISM IN ANIMALS

by

Mikael Puurtinen & Veijo Kaitala 2002

http://users.jyu.fi/~hemipu/1120_Puurtinen.pdf

II

PRIMERS FOR NINE MICROSATELLITE LOCI IN THE HERMAPHRODITIC SNAIL *LYMNAEA STAGNALIS*

by

K. Emily Knott, Mikael Puurtinen & Veijo Kaitala 2003

III

CAN FITNESS EFFECTS OF SELF- AND CROSS-FERTILIZATION EXPLAIN THE NATURAL MATING SYSTEM OF THE HERMAPHRODITIC SNAIL *LYMNAEA STAGNALIS?*

by

Mikael Puurtinen, K. Emily Knott, Susanna Suonpää,
Kari Nissinen & Veijo Kaitala

Submitted manuscript

IV

**THE EFFECTS OF MATING SYSTEM AND
GENETIC VARIABILITY ON SUSCEPTIBILITY
TO TREMATODE PARASITES IN A FRESHWATER
SNAIL, *LYMNAEA STAGNALIS***

by

Mikael Puurtinen, Mirjami Hytönen, K. Emily Knott, Jouni Taskinen,
Kari Nissinen & Veijo Kaitala

Submitted manuscript

V

GENETIC VARIABILITY AND DRIFT LOAD IN POPULATIONS OF AN AQUATIC SNAIL

by

Mikael Puurtinen, K. Emily Knott, Susanna Suonpää,
Tapio van Ooik & Veijo Kaitala 2004

http://users.jyu.fi/~hemipu/1550_Puurtinen.pdf