Ontogeny and Population Biology of a Sex-Limited Colour Polymorphism

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Evolutionary dynamics and population biology of a polymorphic insect

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Abstract

Conspicuous heritable polymorphisms are useful to address the question if morph frequencies are stable or whether they fluctuate between generations. Colour polymorphisms have been studied by ecological geneticists in the past, but there are few long-term studies of genetic dynamics across multiple generations. We studied morph-frequency dynamics and female fecundity in the trimorphic blue-tailed damselfly (*Ischnura elegans*). The morphs include a male-coloured (androchrome) type of female which is thought to be maintained by frequency-dependent sexual conflict. Morph frequencies changed significantly between years across all populations. There was evidence for directional frequency-change since androchrome females increased in 9 out of 10 populations across a four-year period. There was heterogeneity between populations in their evolutionary trajectories, partly caused by population age: androchrome frequencies were initially high in young populations but gradually decreased and approached the level of old populations. We discuss the possible causes of morph-frequency fluctuations, and the role of morph-specific fecundity, dispersal and other forces influencing evolutionary dynamics in this system.

Key words: ecological genetics, frequency-dependence, sex-limited polymorphism, synchrony, sexual conflict, thermoregulation

Introduction

How selection and other forces such as genetic drift, mutation and migration mould patterns of genetic variation in natural populations is a central topic in population genetics and evolutionary biology. These questions attracted considerable interest among early theoretical population geneticists (Fisher 1930; Wright 1931; Lewontin 1974), and they continue to be in focus of current evolutionary studies (Lynch & Walsh 1998). The fitness consequences and extent of adaptive genetic variation in natural populations is of much interest to ecologists and evolutionary biologists, which is exemplified by many recent long-term field studies and reciprocal transplant experiments in different taxa (Mousseau et al. 2000). Several recent empirical studies have demonstrated rapid evolutionary change and the presence of ample adaptive genetic variation in natural populations (Reznick *et al*. 1997; Huey *et al*. 2000; Merilä *et al*. 2001; Grant & Grant 2002).

Before the development of molecular biology, evolutionary biologists interested in genetic variation in natural populations often studied visible marker phenotypes with a known genetic basis (Lewontin, 1974). This was the basis of the mainly British tradition of "ecological genetics" (Ford 1975), although this research approach was also popular in France, North America and Russia (Provine 1986). Examples of study systems include shell-colour patterns in *Cepaea*-snails (Wright 1978), chromosomal inversions in *Drosophila* (Dobzhansky 1970), melanic and non-melanic moths (Haldane 1956; Cook 2003) and pollination polymorphisms and self-incompatability systems in plant

populations (Eckert & Barrett 1995; Ågren & Ericson 1996). The genetic basis of the phenotypic traits in these polymorphic systems is often simple and due to one or a few loci. By following natural populations over multiple generations, researchers could literally observe allele frequency changes directly, by comparing the frequencies of the morphs between years or populations.

Recently, systems with discrete morphs have been subject to increased interest. This increased attention is due to both theoretical and empirical advances. First, the development of the theoretical models for sympatric speciation suggest that discrete morphs, whether they are colour morphs or trophic morphs, could be an important intermediate step in the evolution of reproductive of isolation by sexual or natural selection (Higashi *et al*. 1999; Dieckmann & Doebeli 1999; Takimoto *et al*. 2000). Second, it has been observed in some polymorphic systems that there can be rapid changes in morph frequencies across generations and the genetic composition of the populations may even exhibit cyclical dynamics (Hori 1993; Sinervo & Lively 1996; Moorcroft et al. 1996; Sinervo et al. 2000; Halkka et al. 2001; Horth & Travis 2002; Zvereva et al. 2002; Bell et al. 2004).

There are still relatively few long-term ongoing studies of morph frequency dynamics in natural populations. Notable exceptions are the classical ecological genetic studies, and more recent work on colour polymorphisms in lizards and insects, and some plant species such as the *Linanthus* flower colour polymorphism in California (Schemske & Bierzychudek 2001). In this paper, we present data on morph-frequency changes in 13

populations across four generations in the colour polymorphic European damselfly *Ischnura elegans*. Many *Ischnura*-species contain two or three female colour morphs, which are thought to be maintained by sexually antagonistic male-female mating interactions (Robertson 1985; Cordero 1992; Cordero et al. 1998; Van Gossum et al. 2001). A genetic basis of these colour polymorphisms has been confirmed by breeding experiments in three of the different species of *Ischnura* (Cordero 1990). We have recently developed an explicit population genetic model which shows that multiple female morphs are maintained through frequency-dependent male mating harassment of common female phenotypes, similar to the apostatic survival selection on common prey caused by predators (Svensson et al. 2005).

Up until now, there have been no long-term studies of morph-frequency changes across multiple generations in *I. elegans*. Previous studies on such colour morphs in this and related species have either compared neutral molecular variation with morph-frequency variation between populations to indirectly estimate selection (Andrés et al. 2000), or estimated morph-specific mortalities using mark-resight studies (Cordero 1992; Cordero et al. 1998). Previous studies aiming to investigate morph-fecundities have failed to detect any difference between female morphs (Fincke 1994). Given that sexually antagonistic selection may affect fecundity rather than mortality (Holland & Rice 1999), additional data on fecundity-differences between such colour morphs are clearly needed. In this paper we therefore present data on fecundity-variation between morphs in different populations and years.

The goal of this study was not to test directly whether sexually antagonistic selection is the ultimate cause of the maintenance of this polymorphism. Rather, our starting point is that this polymorphism is maintained by some sort of balancing selection, and we do instead search for signatures of frequency-dependent or other forms of selection, which should be revealed as morph-frequency changes between generations and/or differential female fecundity. We discuss the role of both biotic and abiotic factors that could influence morph-frequency-dynamics in this system.

Material and methods

Natural history of *Ischnura elegans* and genetics of colour morphs

I. elegans is a small damselfly that occurs in Europe from northern Spain to southern Sweden; its distribution also extends further into Eastern Europe and Asia (Askew 1988). It spends one year in the larval stage and emerges as an adult in late May or early June in Sweden (Askew 1988). After emergence, females pass through a series of ontogenetic colour changes over the course of 6-8 days, until they reach sexual maturity when colour is fixed and the morphs are visible and possible to classify (Askew 1988; Robinson & Allgeyer 1996). The three female colour morphs that occur in this species are androchrome females, *infuscans* and *infuscans-obsoleta* (Cordero et al. 1998). Androchromes are blue and have similar patterning and colouration as males: three black bands on the thorax and blue on the thorax and abdomen. *Infuscans*-females have similar patterning as males but olive-green colouration. *Infuscans-obsoleta*-females have only

one black band on the thorax and are pinkish in the immature stage, but their thorax colour becomes increasingly darker and more brownish towards the adult stage. A blue band on the abdomen is present at the adult stage only in androchrome females.

After reaching sexual maturity, males and females are found copulating, often close to water, where oviposition takes place (E. Svensson, pers. observations). Females in *I. elegans* and other *Ischnura* species mate with multiple males (Cooper et al. 1996; Sirot & Brockmann 2001), and copulations can last for 5-6 hours, if not interrupted (Robinson & Allgeyer 1996). Females oviposit alone, in vegetation close to the water's surface (Askew 1988).

The genetic basis of colour polymorphism in *Ischnura* is due to a single mendelian locus, or alternatively, a set of tightly linked loci (Cordero 1990). In the sister species of *I. elegans*, *I. graellsii*, it has been demonstrated via laboratory breeding experiments over three generations that the colour polymorphism is caused by an autosomal locus with three alleles in a dominance hierarchy and with sex-limited expression to females (males are monomorphic) (Cordero 1990). Analyses of hybrid progeny between *I. elegans* and *I. graellsii* indicate that the genetic basis is identical in these two sister species (A. Cordero, pers. comm.). If the three alleles are denoted *p*, *q* and *r*, then A-females are *pp, pq* or *pr*, I-females are *qq* or *qr* and IO-females are *rr*. Thus, the androchrome-allele (*p*) is dominant over both *q* and *r*, the infuscans-allele (*q*) is dominant over *r*, and the *r*-allele is recessive to both *p* and *q*.

Study area and study populations

We studied morph frequency changes, fecundity and population dynamics in a series of damselfly populations in southern Sweden during the summer seasons 2000 to 2003 (four damselfly generations). The study populations, 13 in total, of which 10 were visited in all years, are small shallow ponds, located within an agricultural landscape. One of the most common damselflies in these ponds is *I. elegans.* Other common damselflies in these ponds are *Coenagrion puella, C. pulchellum*, *C. hastulatum* and *Enallagma cyathigerum*. All 13 ponds are geographically isolated from each other, and the pairwise distances between them range from 1.083 to 41.113 km (Mean = 14.54, SE = 0.93, statistics from 78 pairwise comparisons between the ponds). Some of these ponds are old and more or less natural wetlands (Habo Gård, Lomma, Vallby Mosse and Vombs Vattenverk), whereas the other ponds were constructed during the period 1990-1999 as part of an environmental program (Flyinge 30 A1, Flyinge 30 A3, Hofterup, Höje Å 6, Höje Å 7 and Höje Å 14). This program was partly funded by the European Union (EU), and aims to increase biodiversity in the agricultural landscape (see http://www.ekologgruppen.com/htm/start.htm for further information).

Field and laboratory work

We visited the study populations at regular intervals during four summer seasons (June-July) 2000- 2003. During these visits, we caught as many damselflies as possible, and determined sex and age of each individual and, in the case of females, we also classified

them according to colour morph. A number of females were kept for estimation of fecundity and morphometric data-collection, whereas all other females were released at the site of capture. The aim of our regular visits was to get reliable estimates of annual morph frequencies and maximize the detection probability of rare morphs. We were successful in this regard, since most populations that were visited more than once during a year contained all three morphs. When a population was visited only one or a few times in a single year and contained only one or two morphs, it is possible that the rarest morph (*infuscans-obsoleta*) went undetected during these visits, and such populations were excluded from further analyses. The mean number of visits per year (± SE) to the intensively studied populations was 3.41 (± 0.31). The mean number of days between separate population visits was 9.65 ± 0.82 (N = 139 unique visits in total). Multiple captures of the same individuals during a single visit were avoided entirely, since captured individuals were kept in plastic containers during the entire catching session. Multiple captures of the same individuals at different visits are unlikely to affect the results, given the long intervals between our population visits (see above) and the fact that the expected life-span of *I. elegans* in the field is considerable shorter than the average length of these intervals (Corbet 1999).

During 2001-2003, but not during 2000, we also estimated adult population densities by actively catching as many damselflies as possible during sessions that lasted for 30-60 minutes, a field methodology that has been used in previous studies on other *Ischnura*-species (Andrés et al. 2000). Most of these density-estimates are available from sunny days when *I. elegans* is mainly active, and hence these estimates reflect an upper limit of

individual encounter rate in a given population. Multiple density-estimates from a single population visited several times were averaged to minimize measurement error and obtain a more stable measure of long-term density. Population densities in this paper are expressed as number of individuals caught per minute (inds./min.).

Morph frequencies for populations were estimated as the number of each particular morph, divided by the total number of females caught. Since only one of the female morphs (*infuscans-obsoleta*) can be safely classified at the immature stage, and the two other morphs (androchromes and *infuscans*) share a similar ontogenetic colour phase (*violacea*; (Askew 1988), we assumed that the proportion of the these two morphs among the immatures was similar to the morph frequency among the adults. This assumes that mortality among these immature *violacea*-females is random with respect to morph, which seems reasonable given that the mature colour morph that is subject to selection is not expressed and visible at this early ontogenetic stage (Cordero 1992).

During 2001-2003, we used a total of 1000 females from the most intensively studied populations to estimate fecundity of the different morphs in different populations. Females were given unique identification numbers, and set up in small plastic containers (approximately 3 cm in diameter and 3 cm in height) during oviposition. Containers with females were kept in an indoor laboratory (about 20 °C and 12 h light regime) during the entire oviposition period (about three days). Females were not fed during the oviposition period, hence the number of eggs laid by a female will only reflect her food intake during her past recent field situation. To stimulate oviposition, we provided each female with a

piece of wet filter paper, which usually elicits oviposition (82.2 % of females oviposited within 1-2 days). After 2 days, the females were taken out of the container and released at a nearby pond close to the laboratory. The pond was located > 1 km away from the closest source population, making it unlikely that females that oviposited in our laboratory were counted twice in the monitoring of population morph frequencies. The eggs obtained from females were left for an additional day. At day 3, we counted the eggs. Freshly laid eggs are transparent, but after 24 hours fertile eggs turn brown, so waiting until day three facilitated the detection and counting of eggs. The mean number of eggs laid per female was 267.4 (SE = 5.10).

Our fecundity-estimate, although being a cross-sectional fitness component, is likely to be correlated with total reproductive fitness. Previous field studies on damselflies have indeed revealed that fecundity per clutch explains between 10 and 50 % of female total life-time fecundity (Fincke 1986; Corbet 1999). Furthermore, although females are capable of laying multiple clutches under continuous access to food in the laboratory and under favourable weather conditions, the daily survival rates of this small insect in the field are very low (Corbet 1999), particularly in the cold and rainy summers of southern Sweden which is the northern limit of this species' distribution (E. Svensson, unpublished data). Hence, in our study area, many, perhaps even the majority, of females may only survive long enough in the field to lay one batch of eggs. However, it is possible that females that laid eggs during the brief laboratory stay would have allocated their eggs in the field over several different clutches, in which case our fecundity-estimates should

reveal the entire reproductive potential of field-caught females from populations differing in ecology.

Of the females that laid eggs, 89.2 % were old and 10.8 % were immature, judged by the stiffness and dryness of their wings. A GLM with four independent variables revealed that there were significant differences in average female fecundity between populations ($F_{14,801} = 3.491$; $P < 0.001$), years ($F_{2,801} = 4.013$; $P = 0.018$) and morphs ($F_{2,801} = 10.477$; $P < 0.001$), but no effects of female age ($F_{1,801} = 2.343$; $P = 0.13$). Thus, fecundity differences between morphs and populations reported in this paper are not confounded by age-differences between morphs. We aimed to obtain fecundity-estimates for all three morphs in all populations studied, but due to low sample sizes of *infuscans-obsoleta* in some populations and some seasons, we were not always able to achieve this goal. Sample sizes for the different morphs will therefore vary between populations and years.

Statistical analyses, model building and model interpretation

All statistical analyses in this paper were performed using the software STATISTICA, Version 6.0 (Statsoft 2003), with the exception of the repeated-measures analysis, which was performed in PROC MIXED of SAS (SAS Institute 1996). In the repeated-measures analysis, we chose the unstructured covariance structure option after first having tested for compound and autoregressive 1 symmetry (SAS Institute 1996). These two alternative forms of covariance structure were rejected in favour of the unstructured option based on the values of the Akaike Information Criterion (AIC) and the Bayesian Information

Criterion (BIC) (SAS Institute 1996). The repeated-measures analysis was a mixed model in which year entered as a random effect whereas population age was treated as a fixed effect. All statistical tests in this paper are two-tailed, and all variables were normally distributed, unless otherwise stated. Hence, means and standard errors (SE:s) are provided throughout the paper.

When investigating global morph frequency dynamics (change in morph frequencies) across all years and populations, we used generalized linear models with a binomial error structure and logit link function. Models were constructed so that the probability of being a particular morph (1: being that morph; 0 being another morph) was the dependent variable, and population and year were the predictor variables (factors). We performed this analysis first for androchrome females (the most common morph), and the model had the following form:

$$\Pr(\text{Androchrome-female}) = a + b*\text{POP} + c*\text{YEAR} + d*\text{POP}*\text{YEAR} \quad (1)$$

where a is an intercept, b and c are the coefficients of the main effects (population and year) and d is the coefficient of the interaction effect (POP *YEAR). This model can be interpreted in the following way: The POP term will reveal any significant differences in androchrome frequency between populations. The YEAR term will reveal any significant changes in androchrome frequency between years (generations) and hence will reflect global changes in the genetic composition of the populations (i. e. evolutionary changes). Finally, the POP*YEAR term will reveal the existence and degree of synchronicity or

asynchronicity of morph frequency-changes in the different populations. If androchrome frequency-changes are entirely synchronous and identical across all populations, the POP*YEAR term will be weak or non-significant. If the POP*YEAR term is significant, it will reflect the fact that the pattern of androchrome frequency-change between years is at least partly asynchronous and different populations follow different evolutionary trajectories. We also present the model results when the dependent variable is the probability of being one of the other morphs (*infuscans* and *infuscans-obsoleta*). For all these generalized models, we checked for evidence of overdispersion (i. e. stat/d. f. >> 1), but in no case it was found, and hence there was no need to rescale the deviance parameter.

For the fecundity-data, we used general linear models (GLM:s) with female fecundity (number of eggs) as the dependent variable, and population, year, morph and all two-way interactions between these factors as predictor variables. Type-III sums-of-squares (SS) were used, since they are suitable when interaction-effects are of particular interest and when data is unbalanced, compared to type-I or type-II SS (Shaw & Mitchell-Olds 1993) In these models, interaction effects involving morph (POP*MORPH and YEAR*MORPH) will reflect spatial and temporal variation in morph fecundities, a pre-requisite for the maintenance of genetic polymorphisms through spatially and temporally varying selection (Kirkpatrick 1996). The YEAR*MORPH interaction will reflect global changes in morph fecundities between years, changes which could either by driven by abiotic factors such as changing temperature between years or by biotic factors such as an altered degree of intraspecific competition, caused by fluctuating population densities or

variation in morph frequencies. The POP*MORPH interaction indicates whether the fecundities of the different morphs differ between populations, which is expected if it is mainly spatially varying selection that is responsible for the maintenance of a genetic polymorphism (Gillespie 1973).

Results

Morph frequency variation between populations and years

Data on average morph frequencies and morph frequency changes across years are presented in Figs. 1-2. Across all populations and years, there was a decrease in frequency of androchromes, and a parallel increase in frequency of *infuscans* (Fig. 2). This can be seen as a downward movement of the population morph frequencies in the ternary plots in Fig. 1, away from the uppermost apex which represent 100 % androchromes, down towards the lower corners in the same plots, with increasing frequency of *infuscans* and *infuscans-obsoleta*. This decrease in androchrome frequency was most noticeable in Flyinge 30 A1 and 30 A3 (Fig. 1B-C), Hofterupssjön, Höje Å 6 and Höje Å 7 (Fig 1G-I), and Lomma (Fig. 1K). However, it is also worth pointing out that other populations, such as Gunnesbo and Habo Gård (Figs. 1 E-F), Vallby Mosse (Fig. 1 L) and Vombs Vattenverk (Fig. 1 M), changed their morph frequencies less across years and appeared to be more stable.

The generalized linear models confirmed the general picture of changing morph frequencies between generations: there was a significant decrease in the frequency of androchromes and an increase in the frequency of the two other morphs from 2000 to 2003 (Table 1; note that these tests only include the 10 populations which were sampled every year during 2000-2003). The overall frequency of androchrome females in these populations decreased from 82.4 to 66.2 % over this time period, which is partly reflected by the YEAR-term in the generalized linear model (Table 1). To further illustrate this, we compared the morph-frequencies at the end of the study period (2003) with those at the beginning (2000) and tested for the presence of a systematic change using paired t-tests (i. e. morph-frequency in 2003 vs. morph-frequency in 2000). We performed this test for all the 10 populations for which we had a complete series and that were monitored in all four seasons. The long-term decline in androchrome frequencies from 2000 to 2003 was observed in 9 out of the 10 populations and was highly significant (Paired t-test: t = 3.966; d. f. = 9; P = 0.003). However, there was also some heterogeneity between populations in their androchrome frequency changes, as revealed by a significant POP*YEAR-interaction (Table 1).

The general decline in androchrome frequencies was more pronounced in some populations than in others (Figs. 1-2). To further investigate this, we separated the 10 populations which we followed during all four seasons into two different groups based on population age: “young” populations in the new wetlands that were created during 1990-1999 (Figs. 1 B-C, G-J) and “old” populations that were either natural wetlands or that were formed more than two or three decades ago (Figs. 1 F, K-M). Both the rate and

pattern of decline in androchrome frequencies differed between young and old populations (POPULATION AGE * TIME : F = 8.89; *P* = 0.0093; POPULATION AGE * TIME^2: F = 7.92; *P* = 0.013. Den. D.f. = 2; Num. D. f.= 8 for both terms). The androchrome females were more frequent (around 90 %) in the young study populations at the beginning of the time period, whereafter they gradually declined and the frequencies approached the same level as in the older populations (about 66 %; see Fig. 3).

In parallel with the decrease in androchrome frequencies, *infuscans*- and *infuscans-obsoleta*-females almost doubled in frequency from 2000 to 2003, again with significant heterogeneity between populations (Table 1). Across all populations, the frequency of *infuscans* increased from 13.1 to 25.9 % (Fig. 2B), and this long-term increase was significant (Paired t-test: $t = 2.780$; d. f. = 9; $P = 0.021$). The frequency of *infuscans-obsoleta* increased from 4.5 to 7.9 % from 2000 to 2003 (Fig. 2C), although this increase was not significant (Paired t-test: $t = 1.493$; d. f. = 9; $P = 0.17$).

Population densities and morph-fecundities

There was significant variation between years and populations in the density of *I. elegans* (Table 2). Höje Å 6, Höje Å 14 and Lomma had the highest population densities, whereas Flyinge 30 A1, Hofterupssjön and Höje Å 7 had the lowest densities (Fig. 4).

Across all years and populations, the average fecundities of the three morphs differed

significantly (Table 3), being similar for androchrome females and *infuscans-obsoleta* (mean fecundities: 251.9 and 238.3, respectively) and about 15-21 % higher for *infuscans* (mean fecundity: 289.2; see Fig. 5A). However, there were significant differences between years in annual fecundity, and the three morphs changed fecundity differently between years, reflected in a significant MORPH * YEAR interaction (Table 3). *Infuscans-* and *infuscans-obsoleta* females increased their fecundity relative to androchrome females from 2001 to 2003 (Fig. 5B).

Discussion

The colour polymorphism in *I. elegans* shows many signs of being a dynamic polymorphism, in which morph frequencies change significantly between generations (Table 1; Figs. 1-3). It has been suggested that the colour polymorphisms in the genus *Ischnura* and other damselfly species are neutral with respect to selection (Fincke 1994). The hypothesis of selective neutrality has been difficult to test, since there have been no replicated long-term data on morph-frequency changes across multiple generations in different populations. The results from this longitudinal study do not support the neutrality-hypothesis. If genetic drift was responsible for these morph-frequency changes, we would not expect any directional change across the years, as we observed for androchrome and *infuscans*-frequencies (Figs. 2A, B). If the polymorphism was entirely neutral with respect to selection, genetic drift would be the only force acting on these colour morphs, and the frequencies of androchromes would be expected to increase in some populations and decrease in others (Hartl & Clark 1997). This random-walk

prediction from genetic drift contrasts with the general frequency-decrease we actually observed (Fig. 2A).

A more direct indication that the polymorphism is subject to selection comes from the differences in absolute and relative fecundities of the morphs between and within years (Table 3; Fig. 5). Although our fecundity-estimate is only a component of total fitness, we note that the 15-21 % higher fecundity of *infuscans*-females compared to the two other morphs potentially constitutes a very strong fitness-advantage. It is of course possible that this fecundity-advantage is either counteracted by selection during other parts of the life-cycle (e. g. during the larval stage), through other fitness components (e. g. female life-span) or is a temporary advantage that will disappear over coming years. Previous claims that such colour morphs do not differ in fecundity, however, were based on sample sizes of only a few dozen individuals (Fincke 1994). The data in this study do indeed indicate that the polymorphism is subject to selection acting through differential female fecundity. Further evidence against selective neutrality of the colour morphs in *Ischnura* comes from a recent study on *I. graellsi* (the sister species of *I. elegans*), which showed that there was a significant discrepancy between morph-frequency variation between populations and neutral molecular population divergence (Andrés et al. 2000). These data, in combination with the novel findings in this study, suggest a role for balancing selection in the maintenance of this polymorphism.

Long-term changes and synchronous genetic dynamics

The overall decrease in androchrome frequencies and the increase in *infuscans*-frequencies reveal that there is some degree of synchrony across populations in morph-frequency dynamics (Figs. 1-2; Table 1). Which are the causes of these partly synchronous long-term changes? Presumably, they are caused by some abiotic factor(s) that are affecting most populations similarly, e. g. a direct or indirect effect of temperature. A role for temperature is indicated by the fact that the recent summers in Sweden have become progressively warmer over the last decade, and in 2002 and 2003 the summer temperatures were actually the highest over the last 500 years in Europe (Luterberger et al. 2004).

Previous studies on other colour polymorphic insects have shown that different morphs often differ in temperature-related performance (Forsman 2001; Forsman et al. 2002; True 2003), and similar morph-differences may exist in *Ischnura*. Other recent studies on insects have revealed the presence of substantial genetic variation in temperature tolerance within populations (Bradshaw & Holzapfel 2001; Rank & Dahlhoff 2002). In this context, we note that the relative fecundities of *infuscans*- and *infuscans-obsoleta* females increased relative to androchrome females from 2001 to 2003 (Table 3; Fig. 5B). This is consistent with a temperature-related reproductive advantage of these two morphs over androchrome females. The increased relative fecundities of these two other morphs do coincide with the decline of androchrome frequencies over the same time period (Fig. 2A).

We are not aware of any previous studies on synchronous morph-frequency changes or allele frequency changes across multiple populations. The topic of synchrony is not much discussed in population genetics, but ecologists working on population dynamics have often documented synchronous changes in population densities between more or less distant populations (Koenig 1999). Synchrony in population dynamics have been explained either by dispersal or through some global abiotic factors that are affecting all populations more or less equally (Koenig 1999). The relative importance of dispersal and abiotic factors in causing synchrony is still subject to discussion, but most workers seem to agree that dispersal can not be the entire explanation for synchronous population dynamics (Koenig 1999). That there may be some limited role for dispersal in *I. elegans* is indicated by the fact that the pattern of androchrome frequency-decline differs between young and old populations (Fig. 3), which is further discussed below. We are currently investigating the degree of gene flow between our study populations, using AFLP-markers (unpublished data).

Population variation and local frequency-dynamics

Although there were significant changes in morph frequencies between years, there was also heterogeneity between populations in their evolutionary trajectories (Table 1). Some populations changed their morph frequencies substantially, whereas others had more stable frequencies (Figs. 1-3). The significant population term (Table 1) shows that there were differences between populations in their morph frequencies even when controlling for the annual frequency-changes. Such population-differences in morph frequencies

could arise from ecological differences, e. g. differences in densities of *I. elegans* (Fig. 4), densities of other co-existing damselflies (*Enallagma cyathigerum* and *Coenagrion* spp.), or differences in ectoparasite intensities between populations (E. Svensson & A. Coreau, unpublished data).

The differing patterns of androchrome-frequency dynamics in young and old populations (Fig. 3), are interesting since they suggest differential patterns of dispersal between morphs and that new populations may not yet have reached evolutionary equilibrium. Our data indicate that androchrome females may be more prone to disperse and colonize novel areas, leading to higher initial frequencies of this morph in young populations (Fig. 3). Alternatively, some ecological factors may differ between young and old populations that are unrelated to population age *per se*. One such ecological difference is the amount of surrounding vegetation around ponds: newer populations have in general less amount of high vegetation close to the shores (E. Svensson, pers. obs.). However, the rate of decline of androchrome frequencies in young populations (Fig. 3) is too fast to be attributed to these ecological differences, since vegetation differences have not changed at the same rate. We therefore suggest that the differences in androchrome frequency-change between young and old populations is indeed caused by population age *per se*, and is unlikely to be caused by some other ecological factor such as changes in vegetation patterns.

The difference between young and old populations is likely to reflect morph-specific dispersal, but it is also entirely possible that androchrome females, as male-mimics, may

have some sexual selection advantages in the newly founded populations. This androchrome advantage could then decrease over time. For instance, sex-ratios may be more male-biased in newly founded populations, leading to extensive male mating harassment, which may favour androchrome females over the two other morphs. These possibilities, along with potential morphological dispersal-adaptations (e. g. differences in wing length between morphs) will be discussed in more detail in a future paper.

Does frequency-dependence limit population divergence in morph frequencies?

The three female colour morphs in *I. elegans* are apparently present in all populations we have studied to date, and there is no sign that they are lost through local genetic drift. The long-term directional changes we have observed are inconsistent with the action of genetic drift, which would lead to phenotype fixation, loss of morphs and monomorphism in local populations (see discussion above). However, even if genetic drift does not play a major role in this system, the problem of morph-maintenance remains. Any local ecological differences that would favour one of the morphs through frequency-independent selection would ultimately also cause monomorphism. The observed variation in morph-frequencies between populations is consistent with a (minor) role for genetic drift, or frequency-independent selection at the local level, operating against the stronger force of balancing selection that is likely to be present in all populations.

We suggest that negative frequency-dependent selection in favour of the rare morphs counteracts population divergence in morph frequencies. Male mating behaviour fuels

this form of frequency-dependent selection, since as soon as a morph becomes too common in a population, its fecundity will decrease due to excessive male mating harassment (Fincke 2004; Svensson et al. 2005). Increased male mating harassment directed towards common morphs thus generates a form of apostatic and frequency-dependent sexually antagonistic selection that maintains the polymorphism and prevents loss of morphs in local populations (Svensson et al. 2005).

Colour polymorphisms and evolutionary dynamics in ecological time

The results in this study illustrate the value of visual marker phenotypes, such as colour polymorphisms to study evolutionary dynamics. By following multiple populations over time, and thus have both spatial and temporal replication, one can document evolutionary dynamics in the form of both long- and short-term frequency changes (Figs. 1-2) and try to elucidate the various forces forces acting on such polymorphisms. Moreover, when patterns of morph-frequency dynamics differ between populations of different age (Fig. 3), inferences about morph-specific dispersal behaviour and colonization ability can be made. Long-term and population-biological approaches should preferably be integrated with short-term studies aiming to estimate fitness-components such as fecundity (Fig. 5), to obtain more direct evidence for selection on morphs. Such integrative approaches are needed, since much previous work in these systems have only studied one or a few populations at a single point in time and rarely estimated other fitness-components than mortality (see Introduction).

Recently, both theory (Orr & Coyne 1992; Orr 1999) and empirical evidence (Schemske & Bradshaw 1999) indicate that genes of major effect may actually be both important and common. Colour patterns in animals and plants are often genetically correlated with other adaptive traits (Svensson et al. 2001) and are often subject to strong sexual or natural selection (Moorcroft *et al*. 1996; Losey *et al*. 1997; Subramaniam & Rausher 2000); Sinervo and Lively 1996; Sinervo et al. 2000). Colour patterns can often also provide the basis as future species recognition characters following phenotype fixation of different morphs in different allopatric populations (Mather 1955; Lande *et al*. 2001; West-Eberhard 2003). Colour loci thus have many of the characteristics that make them candidates for genes of major effect on fitness and colour polymorphisms should be well-suited to study various questions in adaptive evolution, including evolutionary change on ecological time-scales (this study).

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Table 1 Generalized linear models (binomial error, logit link function) of the probability of belonging to a particular morph (yes = 1; no = 0) as a function of population (POP), year (YEAR) and the POP x YEAR-interaction. Data from 10 populations that were followed during all four seasons (2000-2003). A. Androchrome morph. B. *Infuscans*-morph. C. *infuscans-obsoleta*-morph. For details about sample sizes and particular population trajectories, see Results and Figs. 1-3.

Source	d. f.	Log Likelihood	χ^2	*P*
A. Androchrome morph				
Population (POP)	9	-946.978	36.221	<0.001
Year (YEAR)	3	-942.758	27.782	<0.001
YEAR x POP	27	-954.252	50.770	0.0037
B. *Infuscans* morph				
Population (POP)	9	-830.073	40.542	<0.001
Year (YEAR)	3	-818.632	22.883	<0.001
YEAR x POP	26	-787.371	62.520	<0.001
B. *Infuscans-obsoleta* morph				
Population (POP)	9	-448.502	25.635	0.0023
Year (YEAR)	3	-445.141	6.722	0.081
YEAR x POP	23	-418.405	53.471	<0.001

Table 2 ANOVA for the effects of year and population on density of *I. elegans.* For presentation of Least-Squares mean of the population effect: see Fig. 4. Density-estimates obtained from active capture of damselflies during 30-60 minutes (See Material and Methods). The two-way interaction (Year x Population) was not significant, and hence only a main-effects model is presented. Type-III sums of squares were used. Full model: $F_{11,108} = 2.934$; $R^2 = 0.152$; $P = 0.0020$.

Source	d.f.	MS	F-ratio	*P*
Year	2	1.434	5.340	0.006
Population	9	0.667	2.483	0.013
Error	108	0.269		

Table 3 Two-way ANOVA for the effects of population, morph, year and all two-way interactions on female fecundity in *I. elegans.* For presentation of Least-Squares means of the MORPH and MORPH x YEAR-terms: see Fig. 5. The three-way interaction (POP x MORPH x YEAR) was not significant. Type-III sums of squares were used. Full model: $F_{53,656} = 2.825$; $R^2 = 0.120$; $P < 0.001$.

Source	d.f.	MS	F-ratio	*P*
Population (POP)	9	34894	2.153	0.024
Morph (MORPH)	2	76433	4.704	0.0094
Year (YEAR)	2	42000	2.585	0.076
POP x MORPH	18	20684	1.273	0.20
POP x YEAR	18	29142	1.794	0.023
MORPH x YEAR	4	80977	4.983	0.0006
Error	656	16248		

LEGENDS TO FIGURES

Fig. 1 Ternary plots showing morph-frequency changes across four seasons in 13 different populations of *Ischnura elegans*. Letters (A-M) refer to different populations that were followed during at least three of the four seasons 2000-2003: A. Fjelie (66), B. Flyinge 30 A1 (84), C. Flyinge 30 A3 (193), D. Genarp (196), E. Gunnesbo (84), F. Habo Gård (238), G. Hofterup (121), H. Höje Å 6 (183), I. Höje Å 7 (106), J. Höje Å 14 (228), K. Lomma Kyrkdamm (379), L. Vallby Mosse (135) and M. Vombs Vattenverk (293). Sample size (total no. females) within parenthesis after each population. Each apex in the ternary plot shows the situation when a population is entirely composed (i. e. 100 %) of a particular morph (A = androchromes, I = *infuscans*, IO = *infuscans-obsoleta*). Vertices show dimorphic populations/situations, and all data points within the ternary plots are trimorphic populations/situations. Solid black dot shows morph frequency of the first season (2000), open dots show subsequent seasons.

Fig. 2 Detailed population trajectories and morph-frequency changes in the 10 most intensively studied populations that were followed during all four seasons (2000-2003). A. Androchrome frequencies, B. *infuscans* frequencies, C. *infuscans-obsoleta* frequencies. For sample sizes; see legend to Fig. 1.

Fig. 3 Percentage of androchrome females in populations of different age across the entire study period (2000-2003; four generations). “Young” populations (N = 6) are populations in wetlands created during 1990-1999, whereas “old” populations (N = 4) are

either natural wetlands or wetlands created more than two decades ago. The pattern and rate of decline in percentage of androchromes differs significantly between the two population categories (see Results).

Fig. 4 Mean population densities (± SE:s) of *I. elegans* in 10 different populations during 2001-2003. For statistical tests of significance: see Table 2. Sample sizes (no. unique visits to estimate densities) below each population estimate.

Fig. 5 Female fecundity in relation to colour morph, population and year. Least-square means and 95 % confidence limits are shown. Sample sizes (no. females) below each estimate. For statistical tests; see Table 3. A. Differences between colour morphs across all years and populations. B. Differences between years for different morphs across all populations.

Fig. 1

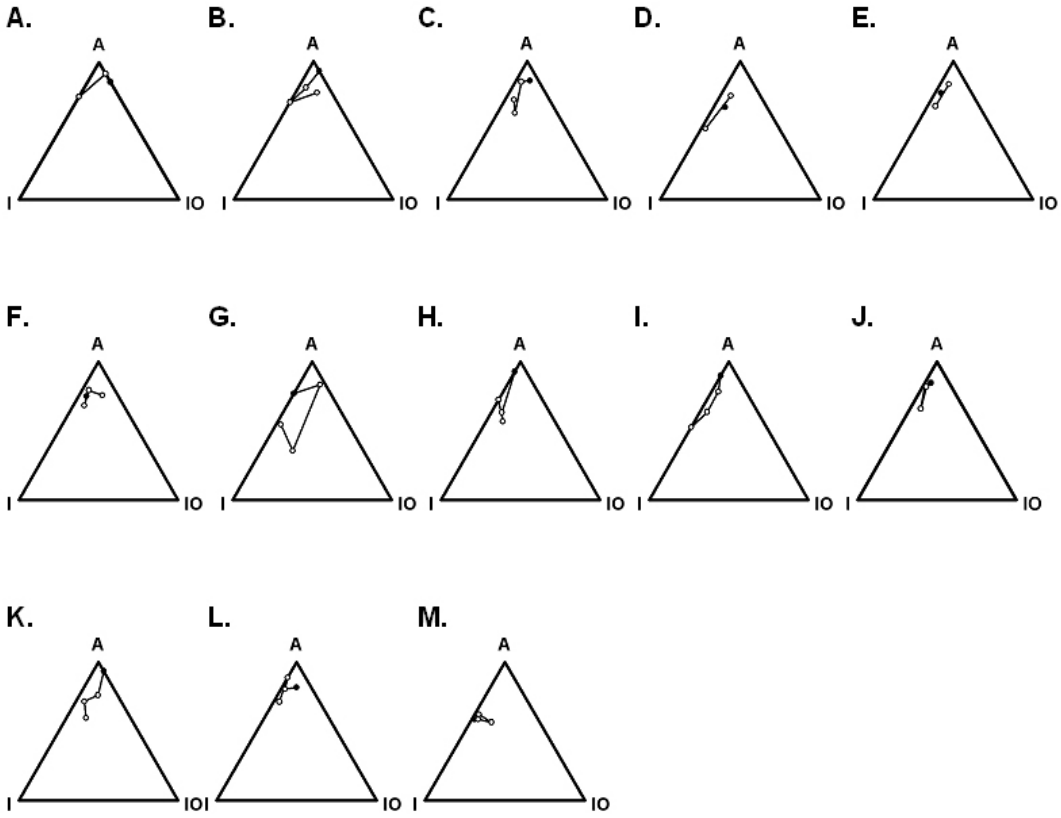


Fig. 2

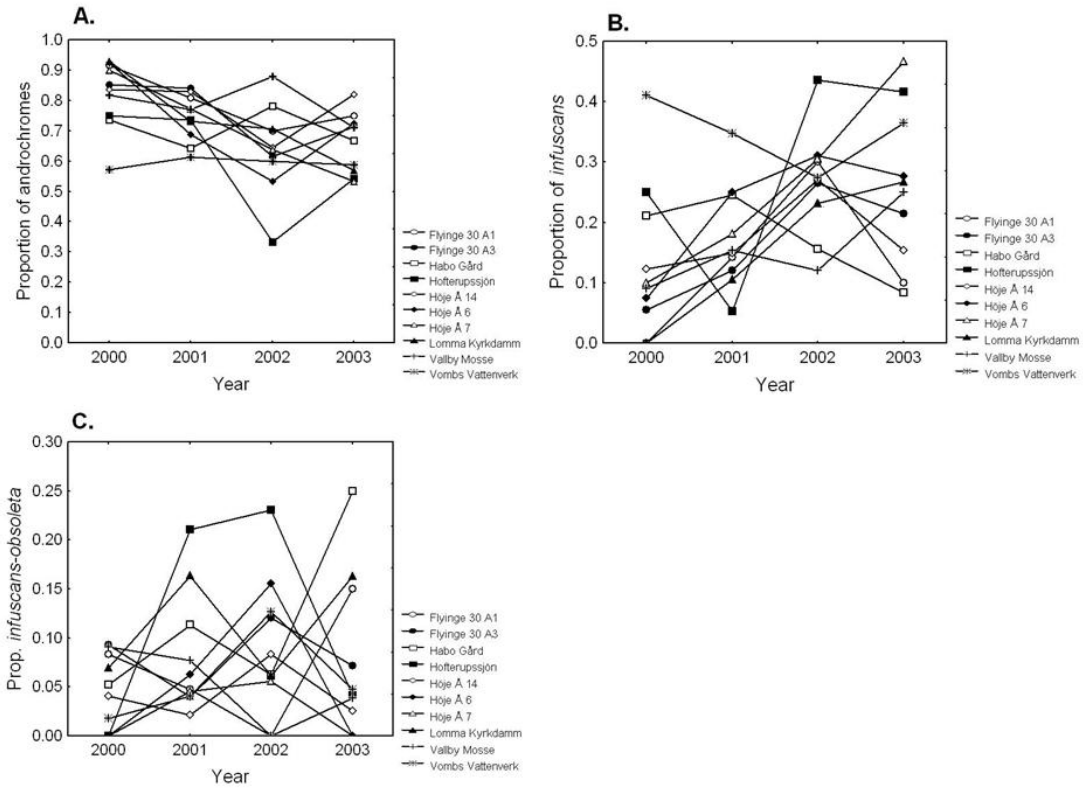

A.
Proportion of androchromes
Year
B.
Proportion of infuscans
Year
C.
Prop. infuscans-obsoleta
Year
Flyinge 30 A1
Flyinge 30 A3
Habo Gård
Hofterupssjön
Höje Å 14
Höje Å 6
Höje Å 7
Lomma Kyrkdamm
Vallby Mosse
Vombs Vattenverk

Fig. 3

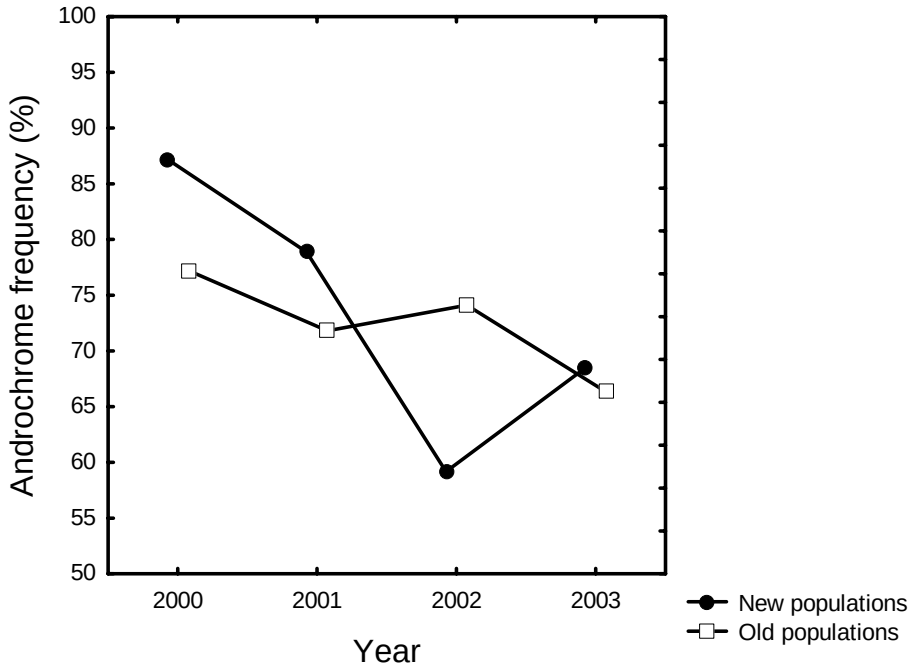

Androchrome frequency (%)
100
95
90
85
80
75
70
65
60
55
50
2000
2001
2002
2003
Year
New populations
Old populations

Fig. 4

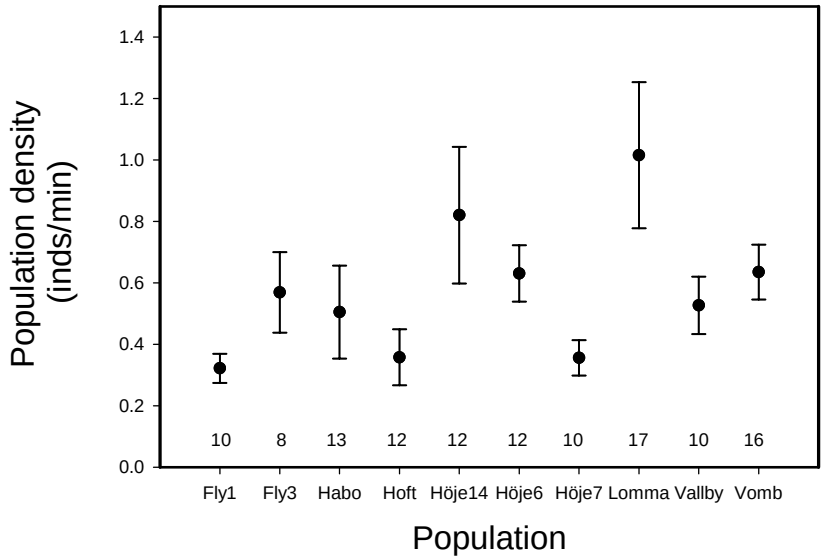
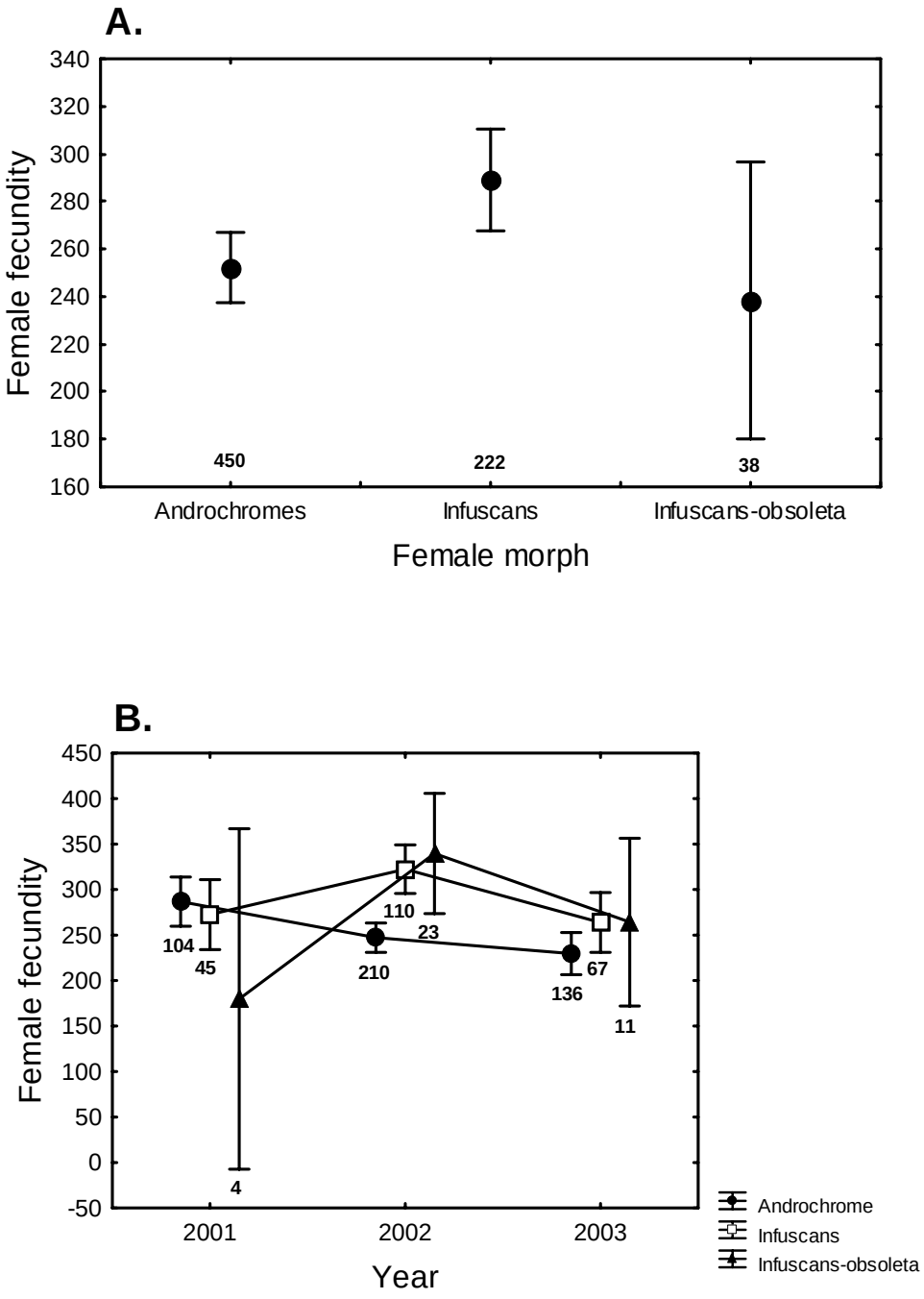

Population density
(inds/min)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10
8
13
12
12
12
10
17
10
16
Fly1
Fly3
Habo
Hoft
Höje14
Höje6
Höje7
Lomma
Vallby
Vomb
Population

Fig. 5


A.
Female fecundity
340
320
300
280
260
240
220
200
180
160
450
222
38
Androchromes
Infuscans
Infuscans-obsoleta
Female morph
B.
Female fecundity
450
400
350
300
250
200
150
100
50
0
-50
104
45
4
210
110
23
136
67
11
2001
2002
2003
Year
Androchrome
Infuscans
Infuscans-obsoleta