Patterns of differentiation in a colour polymorphism and in neutral markers reveal rapid genetic changes in natural populations

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ABSTRACT

The existence and mode of selection operating on heritable adaptive traits can be inferred by comparing population differentiation in neutral genetic variation between populations (often using F_{st}–values) with the corresponding estimates for adaptive traits. Such comparisons indicate if selection acts in a diversifying way between populations, in which case differentiation in selected traits is expected to exceed differentiation in neutral markers (F_{st}(selected) > F_{st}(neutral)), or if negative frequency-dependent selection maintains genetic polymorphisms and pulls populations towards a common stable equilibrium (F_{st}(selected) < F_{st}(neutral)). Here we compared F_{st}-values for putatively neutral data (obtained using AFLP) with estimates of differentiation in morph frequencies in the colour-polymorphic damselfly *Ischnura elegans*. We found that in the first year (2000), population differentiation in morph frequencies was significantly greater than differentiation in neutral loci, while in 2002 (only two years and two generations later), population differentiation in morph frequencies had decreased to a level significantly lower than differentiation in neutral loci. Genetic drift as an explanation for population differentiation in morph frequencies could thus be rejected in both years. These results indicate that the type and/or strength of selection on morph frequencies in this system can change substantially between years. We suggest that an approach to a common equilibrium morph frequency across all populations, driven by negative frequency-dependent selection, is the cause of these temporal changes. We conclude that inferences about selection obtained by comparing F_{st}-values from neutral and adaptive genetic variation are most useful when spatial and temporal data is available from several populations and time points and when such information is combined with other ecological sources of data.

INTRODUCTION

Comparing population differentiation of neutral loci and loci presumed to be subject to selection is a common way to indirectly infer the operation of selection in natural populations (McKay & Latta 2002), for instance by comparing F_{st}-values for neutral loci with those for loci suspected to be subject so selection (Lynch & Walsh 1998). If F_{st}(selected) > F_{st}(neutral) then populations show greater differentiation than expected by genetic drift, which can be a result of adaptation to local environmental conditions (Lynch & Walsh 1998). If F_{st}(selected) < F_{st}(neutral) then populations show less differentiation in adaptive traits than expected by drift, indicating that similar selection pressures are preserving trait values over an extended geographical area (Lynch & Walsh 1998). This latter pattern may occur when negative frequency-dependent selection maintains a genetic polymorphism at a common stable equilibrium shared by a number of populations (Andrés, Sánchez-Guillén, & Cordero Rivera 2000). Finally, when F_{st}(selected) = F_{st}(neutral), population differentiation in the trait of interest does not exceed the expectation from genetic drift. Indirect studies of selection of this kind are particularly useful in the context of discrete heritable polymorphisms since some sort of balancing selection is usually considered necessary to maintain such polymorphisms over evolutionary time (Mazer & Damuth 2001), and the genetic basis of the polymorphism is often known (Andrés, Sánchez-Guillén, & Cordero Rivera 2000; Cameron 2001; Jorgensen, Richardson, & Andersson 2006; Kärkkäinen, Løe, & Ågren 2004; Schemske & Bierzychudek 2001).

Here, we apply this analytical approach to the colour-polymorphic damselfly *Ischnura elegans*, in order to infer if this polymorphism is subject to selection. Males of *I. elegans* are monomorphic, but females may belong to one of three distinct phenotypic morphs: the male-

like Androchrome morph, or one of the two more cryptic morphs, Infuscans and Infuscans-obsoleta (Corbet 1999). Previous field studies have suggested that the morphs are subject to negative frequency-dependent selection caused by male mating harassment (Gosden & Svensson 2007; Svensson, Abbott, & Härdling 2005). The more common a morph is in the population, the more it is harassed by males, resulting in decreased female fecundity of common morphs (Svensson, Abbott, & Härdling 2005). In addition, the morphs differ in morphology, development time, and fecundity (Abbott & Svensson 2005; Abbott 2006; Svensson & Abbott 2005; Svensson, Abbott, & Härdling 2005), suggesting that the female morphs are phenotypically integrated alternative strategies. Given these morph-specific differences, it is possible that each morph exploits a slightly different ecological niche. If population differentiation in morph frequencies is found to be greater than expected from genetic drift, this pattern may reflect local adaptation to differing environmental conditions. On the other hand, if negative frequency-dependent selection operates on this polymorphism, the theoretical expectation at equilibrium would be that population differentiation in morph frequencies should be less than expected from genetic drift (Andrés, Sánchez-Guillén, & Cordero Rivera 2000). Since populations of this species show continual and rapid change in morph frequencies (Svensson, Abbott, & Härdling 2005) they may be approaching a common equilibrium determined by negative frequency-dependent selection, but on different population-specific trajectories. If this is the case, then population differentiation may be greater than expected from drift despite the fact that the equilibrium value is similar in all populations.

Although both diversifying and homogenizing selection have been inferred in other polymorphic damselfly species in the past (Andrés, Sánchez-Guillén, & Cordero Rivera 2000; Wong, Smith, & Forbes 2003), these previous studies have either relied on single point

estimates in time and/or else used relatively few focal populations (between 2 and 5). Our study differs from these previous studies in that we have both compared more populations (12) and replicated our study across two years (2000 and 2002), a period of three generations. Interestingly, we found that despite being only two years apart, our inferences about selection at each point changed substantially over this time period. We suggest that this is because our study populations have not yet reached their evolutionary equilibria. Non-equilibrium dynamics of this kind may, however, be a general feature of natural populations of both this and other species. Our results will therefore have general implications for the utility of indirect inferences of selection, which is currently a popular research approach among evolutionary biologists and molecular ecologists (see references above).

MATERIALS AND METHODS

Field work and study organism

Our study took place in a series of populations of *Ischnura elegans* in southern Sweden (Fig. 1), which is at the northern end of its distributional range in Europe (Askew 1988). This damselfly species is univoltine in Sweden, with one non-overlapping generation per year (Corbet 1999). As discussed above, *I. elegans* has three female morphs, one of which (the Androchrome morph) is a male mimic (Askew 1988; Svensson, Abbott, & Härdling 2005). Morph identity in *Ischnura elegans* is controlled by a single locus with 3 alleles in a dominance hierarchy, and with expression sex-limited to females (Sánchez-Guillén, Van Gossum, & Cordero Rivera 2005). The dominance-hierarchy of the morph alleles is linear, with the Androchrome allele (denoted by "A") dominant over the two other alleles (denoted by "I" for Infucscans and "IO" for Infuscans-obsoleta), i. e. A > I > IO (Sánchez-Guillén,

Van Gossum, & Cordero Rivera 2005). A population composed of only the Androchrome phenotype, if it were found, could therefore still contain alleles of the two other morphs, which would be carried by heterozygotes.

Male and female *Ischnura elegans* were captured and collected from 12 study populations outside Lund, in southern Sweden (Flyinge 30A1, Flyinge 30A3, Genarp, Gunnesbo, Habo, Höje å 6, Höje 7, Höje å 14, Lomma, Vallby, and Vombs vattenverk; Fig. 1). Of these populations, several are located in recently artificially created wetlands (Flyinge 30A1, Flyinge 30A3, Höje å 6, Höje 7, and Höje å 14) while others are either naturally-occurring or else artificially created but long-established ponds (age >20 years at the time of sampling; Genarp, Gunnesbo, Habo, Lomma, Vallby, and Vombs vattenverk). Field work took place from the end of May until the beginning of August using hand-held nets in the summers of 2000 and 2002. All females were classified with respect to morph. For more details on field data procedures, see Svensson & Abbott (2005) and Abbott (2006). Individuals used in genetic analyses were stored in ethanol in small plastic tubes. We sampled between 8 and 34 individuals for genetic analysis (mean±SD: 20.61±7.30), and between 12 and 109 individuals for calculation of morph frequency differentiation (mean±SD: 53.44±28.45) from each population in each year. Although southern European populations of *I. elegans* may systematically vary in morph frequencies over the summer (Cordero 1992), this is unlikely to be a problem here. Previous analysis on these and other study populations shows that though the female morphs differ significantly in emergence time, the difference is only about 3 days (Abbott & Svensson 2005). These study populations were sampled repeatedly over typically much longer periods (mean±SD: 31.17±18.31 days).

Laboratory work, molecular genetic analyses, and statistics

Amplified Fragment Length Polymorphism (AFLP) was carried out as described in Vos *et al.* (1995). Ten different primer combinations were tested, and three selected for final analysis: E_{TCG} and M_{CGG}, E_{TAG} and M_{CGC}, E_{TAG} and M_{CGAC}. Samples were run using gel electrophoresis and 46 polymorphic sites were scored for presence/absence of bands by JA and checked blindly by TG. Many more polymorphic sites were evident on the polyacrylamide gels, but only 46 were deemed suitable for analysis. This is because *I. elegans* appears to have a relatively large genome (Staffan Bensch, personal observation), resulting in the production of many bands located too close together for accurate scoring. Data was analyzed using Arlequin (Schneider, Roessli, & Excoffier 2000). To obtain an error rate due to the amplification and electrophoresis steps (Bonin et al. 2004), 14 individuals were amplified and scored twice. The error rate for these steps was determined to be ca. 4.1%, which is comparable to that found in other studies (Bonin et al. 2004 and references therein). Unfortunately, we were unable to determine an error rate for the extraction step since entire individuals were used during extraction, making it impossible to later repeat this step on the same individual. Samples were not analyzed in year- or population-batches to avoid confounding effects due to lab artefact.

For morph frequency differentiation, we calculated morph allele frequency estimates for each population and year from phenotypic morph frequencies using the Hardy-Weinberg formula (Hartl & Clark 1997)**,** and then calculated F_{st}-values based on the estimated allele frequencies. This approach was also used by Andrés, Sánchez-Guillén, & Cordero Rivera (2000) in a similar study.

Due to small and highly fluctuating population sizes, three populations could not be sampled in both years. Because of this, we first analysed the results from each year separately, and then carried out a two-way ANOVA with Type of data (AFLP or Morph) and Year (2000 or 2002) as factors on a reduced data set with 9 populations that had been sampled in both years. For this analysis, a significant effect of Type would indicate that populations had higher overall differentiation in one or the other type of data (for example, consistently higher differentiation in morph frequencies than at neutral loci). A significant effect of year would indicate that populations had higher overall differentiation in one year (for example if differentiation decreased over time). A significant interaction effect would indicate that the effect of type of data was dependent on year. We also checked the robustness of our results to low sample sizes, by testing for differences between neutral and morph frequency data using a subset of the data where populations with small sample sizes for either measure ($\leq$15 individuals) were excluded. This reduced data-set included a total of 6 populations (Flyinge 30A3, Genarp, Habo, Höje å 6, Lomma, and Vomb). To see if changes in differentiation between years were due to moderate changes in all populations, or large changes in just a few populations, we also calculated F_{st}-values for differentiation between years within populations. Since F_{st}-values are calculated in a pairwise way they are not independent, so significance testing and calculation of means was carried out using resampling procedures (permuation tests and bootstrapping) in the program Resampling Stats (Simon 2000).

Although changes in morph frequencies in these populations have been previously analysed as part of a larger data set (Svensson & Abbott 2005), we also carried out a separate analysis of morph frequency changes in these particular populations and years, in order to try to directly relate changes in F_{st}-values to changes in morph frequencies. Because the frequencies of the three morphs are not independent, we decided to analyse changes in

Androchrome frequency only. This is because Androchromes are the most common morph, and therefore provide the most reliable morph frequency estimates, and also because previous analysis indicated that Androchromes had decreased in frequency over the study period (Svensson & Abbott 2005). We therefore tested for changes in mean Androchrome frequency and in the variance in Androchrome frequencies between years using a weighted one-way ANOVA, with weighting according to the number of individuals captured in the population, and degrees of freedom equal to one less than the number of populations in the analysis.

RESULTS

For the full data set, population differentiation in morph-frequencies was significantly greater than population differentiation for the AFLP-markers in the year 2000 (P=0.004), but not significantly different from population differentiation for the same AFLP-markers in 2002 (P=0.166). However, if populations with small sample sizes (≤15) are excluded, population differentiation in morph frequencies was significant for both years (2000: P=0.003; 2002: P<0.001) which strongly suggests that the lack of a significant effect in 2002 may be due to estimation errors from small population sample sizes. Thus, population differentiation in morph frequencies differed significantly from the neutral expectation in both seasons, although the direction of the difference reversed between years (Fig. 2).

To investigate if these changing patterns of differentiation arose from qualitatively different temporal dynamics of the two kinds of markers (i. e. morph-data and AFLP-data), we performed a two-way ANOVA with Type of data (morph or AFLP), year (2000 and 2002) and their interaction as independent variables. There were no significant main effects of Type of data or Year on population differentiation (both P>0.1), but there was a significant

interaction effect (Type*Year: $F_{1, 144}$=13.41, P<0.001). Thus, population differentiation changed significantly between years, but in qualitatively different ways for the two types of markers (Fig. 2). Population differentiation in morph frequencies decreased from 2000 to 2002 (P=0.028, Fig. 2), while differentiation at neutral loci (AFLP) increased over the same time period (P<0.001, Fig. 2). F_{st}-values used in these analyses are shown in Table 1. More evidence of qualitatively different dynamics for neutral genetic data and morph frequency data comes from analysis of the amount of differentiation between years within populations. For neutral data, there are approximately equal amounts of differentiation between years in each population (Table 2), and there is very little difference in mean differentiation between new and old populations (new: 0.039, old: 0.044). In contrast, morph frequency differentiation between years is very large in some populations (e.g. Flyinge 30A1, Höje å 6), and very small in others (e.g. Genarp, Habo), and mean differentiation is much higher in new populations than in old (new: 0.148, old: 0.020; Table 2).

Mean Androchrome frequency across all populations decreased significantly between 2000 and 2002 (P=0.030, Fig. 3) as did the between-population variance in Androchrome frequencies (Levene's test: P<0.0001, Fig. 3). This suggests that the temporal change in morph frequency differentiation was largely a result of changes in frequency of the most common female morph, the Androchromes.

DISCUSSION

Although comparing differentiation at neutral loci with differentiation in traits presumed to be under selection has been used extensively by plant biologists (Jorgensen, Richardson, & Andersson 2006; Kärkkäinen, Løe, & Ågren 2004)**,** relatively few studies of animals have

been carried out to date (e.g. Andrés, Sánchez-Guillén, & Cordero Rivera 2000). Similar studies on other polymorphic damselfly species (Andrés, Sánchez-Guillén, & Cordero Rivera 2000; Wong, Smith, & Forbes 2003) have revealed conflicting results. In one case differentiation in morph frequencies was found to be greater than expected from drift (Wong, Smith, & Forbes 2003), and in another study on a sibling species of *I. elegans* (*I. graellsii*), morph frequency differentiation was found to be smaller than expected from drift (Andrés, Sánchez-Guillén, & Cordero Rivera 2000). The latter result is actually what is expected if negative frequency-dependent selection on this female polymorphism maintains all morphs in all populations (Andrés, Sánchez-Guillén, & Cordero Rivera 2000). Finally, some other recent studies on polymorphic invertebrates (the scarlet tiger moth *Callimorpha dominula*, and the candy-stripe spider *Enoplognatha ovata*) have found that both drift and selection influence morph frequency fluctuations between generations (O'Hara 2005; Oxford 2005).

Interestingly, indirect inferences about selection based on our results varied between years. Population differentiation in morph frequencies was initially (in 2000) significantly higher than at neutral loci (Fig. 2), which is consistent with divergent selection and local adaptation as a cause of population differentiation in this polymorphism. However, only two generations later (in 2002), differentiation in morph frequencies was significantly lower than differentiation at neutral loci, which may result if morph frequencies are rapidly converging to a common equilibrium. This pattern could also be produced if selection pressures due to abiotic factors vary stochastically, with the scale of selection varying from local to regional between years, and with no or weak net selection in some years. However, we believe that an ongoing approach to equilibrium is the more likely scenario, for reasons outlined below. If negative frequency-dependence causes morph frequencies to converge on the same equilibrium frequency and each population approaches along a different trajectory, this will

result in high differentiation in morph frequencies at the start of this process and low differentiation at the end. Our results would therefore demonstrate movement towards a stable equilibrium morph frequency across our study populations.

In order to confirm that our study populations have undergone this process, we would ideally need data from additional years to determine whether populations have in fact now reached a stable equilibrium or if patterns of differentiation fluctuate wildly between years. Although data on morph frequencies are available from 2000 onwards, individuals were only sampled for genetic analysis in 2000 and 2002 because large changes in the neutral population differentiation (Fig. 2) were not expected when we started this study. A significant increase in neutral differentiation over this short time period is surprising, and shows (Fig. 2) that these populations are unlikely to be in equilibrium for either their neutral markers or their morph frequencies. For example, we have observed that in our study area in southern Sweden, newly established populations of *I. elegans* are subject to frequent extinctions and re-colonizations (E. I. Svensson, unpublished data), which is expected to affect patterns of neutral genetic differentiation between populations (Ingvarsson, Olsson, & Ericson 1997). Sexual selection in this species also appears to be strong, since males engage in "scramble" competition (Andersson 1994; Corbet 1999), and there is evidence of temporal variation in the strength and direction of sexual selection on male body size (Gosden & Svensson, submitted). Both these processes (i. e. extincition-recolonization dynamics and sexual selection) should result in consistently small effective population sizes, which will act to increase the importance of genetic drift to neutral population differentiation (Lynch & Walsh 1998). Measures of neutral differentiation between years in each population also suggest small effective population sizes, since there are consistently large amounts of neutral differentiation between years within populations (Table 2).

Several of our study populations are located in recently artificially created wetlands (Svensson & Abbott 2005), and such newly colonized ponds may, due to random colonization by *I. elegans*, start off with very different morph frequencies, i. e. founder effects. Moreover, genotype-specific dispersal (Garant et al. 2005) or differential colonization ability of the morphs according to site could also lead to overrepresentation of certain morphs in new populations, although there is little direct evidence of morph-specific dispersal (Conrad et al. 2002). There is, however, indirect evidence of morph-specific dispersal from patterns of Androchrome frequency changes in new and old populations (Svensson & Abbott 2005). Newly colonized populations have higher Androchrome frequencies during early establishment phases, while these frequencies decline and approach the levels of old populations over time (Svensson & Abbott 2005). In addition, measures of differentiation in morph frequencies between years in each population show that new populations have higher mean differentiation between years than old populations (Table 2), consistent with the result that morph frequencies are changing more rapidly between years in new populations. Colonization of newly-established ponds in combination with morph-specific dispersal and/or frequent recolonizations could potentially explain why population differentiation in morph frequencies was initially greater than expected from drift. After colonization, negative frequency-dependent selection could then act on these populations to bring them closer to a common equilibrium frequency.

Despite the paucity of neutral genetic data, field data on morph frequency changes in these and other populations over several years (Svensson & Abbott 2005) can provide some supporting evidence for the approach to a common equilibrium hypothesis. Analysis of morph frequencies in the 12 populations which are the focus of this study confirmed that both

the frequency of Androchromes and the variance in Androchrome frequency decreased over time (Fig. 3). The observed decrease in the variance in Androchrome frequencies is clearly consistent with a decrease in overall differentiation in morph frequencies (Fig. 2). In a longer longitudinal study, Svensson and Abbott (2005) found that Androchrome frequencies decreased in most populations over a four-year period. Androchrome frequencies in these study populations during this period were typically between 60% and 90%, which is higher than frequencies reported elsewhere in Europe (Italy: 55% Androchromes, Cordero Rivera & Andrés 2001; Ukraine: 24% Androchromes, Gorb 1999).

Thus, morph frequencies in our study populations may be in the process of approaching an equilibrium that is closer to the lower frequency of Androchromes in more southerly populations. At this point, we can not rule out the possibility that equilibrium frequencies also differ geographically. However, an approach to a low-Androchrome equilibrium frequency is also supported by a population genetic model based on fecundity data to estimate frequency-dependent selection (Svensson, Abbott, & Härdling 2005). Results from population genetic modelling and simulations indicate that the equilibrium frequency of Androchromes may be substantially lower than the frequencies that we observed at the onset of our study in 2000 (Svensson, Abbott, & Härdling 2005). These independent lines of evidence all suggest that an ongoing approach to a common equilibrium frequency.

An important assumption to inferences about the existence of selection from comparisons with molecular data, is that the study populations have reached their evolutionary equilibria. As we have discussed above, this is unlikely to be true in our case. However, indirect inferences about the action of selection, such as this study, are still valuable, particularly when combined with additional ecological information, e. g. measurements of fitness

differences between morphs or genotypes, information about dispersal and gene flow, and longitudinal population studies (Abbott & Svensson 2005; Svensson & Abbott 2005; Svensson, Abbott, & Härdling 2005). Our results thus demonstrate the importance of sampling as many populations and time points as possible when studying non-equilibrium systems, and should hopefully stimulate future research in this area.

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415

Table 1: F_{st}-values for morph frequencies and neutral loci in the years 2000 and 2002. Some populations were not sampled in both years, and absent values are marked by a "-". Neutral F_{st}-values were obtained from the analysis of 46 AFLP loci, while morph frequency F_{st}-values were obtained from allele frequency estimates calculated from phenotypic counts. A: Neutral differentiation in 2000. B: Neutral differentiation in 2002. C: Morph frequency differentiation in 2000. D: Morph frequency differentiation in 2002. Abbreviations are as follows: F1 = Flyinge 30A1, F3 = Flyinge 30A3, Ge = Genarp, Gu = Gunnesbo, Ha = Habo, Hof = Hofterups, H6 = Höje å 6, H7 = Höje å 7, H14 = Höje å 14, L = Lomma, Va = Vallby, and Vo = Vomb. Note that negative numbers simply denote an absence of differentiation, and not negative differentiation. Values that are significantly different from zero are in bold.

A)

	F1	F3	Ge	Gu	Ha	Hof	H6	H7	H14	L	Va
F3	**0.035**										
Ge	**0.031**	0.010									
Gu	0.018	**0.031**	**0.027**								
Ha	0.017	-0.004	**0.022**	**0.045**							
Hof	**0.020**	-0.0002	0.011	**0.052**	0.004						
H6	0.001	0.003	0.012	0.020	0.001	0.008					

H7	-	-	-	-	-	-	-				
H14	**0.039**	0.017	0.018	0.028	0.010	0.019	0.006	-			
L	0.012	0.004	0.009	0.016	-0.017	**0.017**	0.004	-	0.022		
Va	-	-	-	-	-	-	-	-	-	-	
Vo	0.001	0.009	**0.016**	**0.045**	0.006	0.011	0.016	-	**0.041**	0.005	-

B)

	F1	F3	Ge	Gu	Ha	Hof	H6	H7	H14	L	Va
F3	**0.068**										
Ge	**0.105**	**0.028**									
Gu	0.025	0.018	**0.047**								
Ha	**0.093**	**0.029**	**0.022**	0.027							
Hof	-	-	-	-	-						
H6	**0.094**	**0.021**	**0.017**	**0.031**	**0.021**	-					
H7	**0.101**	**0.023**	0.007	**0.043**	0.014	-	-0.001				
H14	**0.054**	**0.017**	**0.024**	**0.033**	**0.043**	-	**0.021**	0.018			

L	**0.057**	**0.024**	0.012	**0.037**	-0.002	-	0.011	-0.003	0.018		
Va	**0.112**	**0.021**	**0.053**	**0.065**	**0.059**	-	**0.028**	**0.041**	**0.049**	**0.037**	
Vo	**0.114**	**0.023**	**0.028**	**0.060**	0.015	-	**0.025**	0.015	**0.037**	0.012	**0.031**

C)

	F1	F3	Ge	Gu	Ha	Hof	H6	H7	H14	L	Va
F3	-0.027										
Ge	**0.102**	**0.059**									
Gu	0.064	0.031	-0.017								
Ha	0.053	0.018	-0.026	-0.051							
Hof	**0.236**	**0.180**	0.046	0.008	0.011						
H6	0.092	**0.104**	**0.115**	0.056	0.057	0.066					
H7	-	-	-	-	-	-	-				
H14	0.011	0.005	0.021	-0.018	-0.027	0.064	0.035	-			
L	-0.053	0.023	**0.160**	**0.131**	**0.124**	**0.303**	**0.113**	-	0.059		
Va	-	-	-	-	-	-	-	-	-	-	

Vo	**0.174**	**0.129**	0.013	-0.001	-0.004	-0.013	**0.109**	-	0.054	**0.223**	-

D)

	F1	F3	Ge	Gu	Ha	Hof	H6	H7	H14	L	Va
F3	**0.112**										
Ge	**0.097**	-0.002									
Gu	0.053	0.008	-0.014								
Ha	**0.105**	0.015	-0.014	-0.011							
Hof	-	-	-	-	-						
H6	**0.139**	-0.012	0.015	0.028	**0.039**	-					
H7	0.030	-0.002	-0.004	-0.015	0.010	-	0.007				
H14	**0.073**	-0.013	-0.010	-0.010	0.004	-	-0.007	-0.020			
L	**0.065**	0.003	-0.012	-0.016	-0.006	-	0.019	-0.013	-0.011		
Va	0.027	**0.118**	**0.064**	0.030	0.051	-	**0.163**	0.056	**0.083**	0.049	
Vo	**0.118**	-0.011	0.001	0.011	0.018	-	-0.013	0.001	-0.011	0.006	**0.123**

Table 2: F_{st}-values between years within each population for morph frequencies and neutral loci, in relation to population age. Populations with data missing in one year are excluded. For neutral loci, differentiation between years is similar across populations, and does not appear to be related to population age (mean new: 0.039, mean old: 0.044). For morph frequencies, differentiation between years varies across populations, and mean differentiation is much higher in new populations than in old (new: 0.148, old: 0.020). For details about classification of populations as new and old, see Materials and Methods. Note that negative numbers simply denote an absence of differentiation, and not negative differentiation. Values that are significantly different from zero are in bold.

Population	Neutral data	Morph frequencies	Population age
Flyinge 30A1	**0.104**	**0.289**	New
Flyinge 30A3	0.015	**0.065**	New
Genarp	**0.018**	-0.010	Old
Gunnesbo	**0.056**	-0.032	Old
Habo	**0.070**	-0.032	Old
Höje å 6	-0.010	**0.214**	New
Höje å 14	**0.048**	0.025	New
Lomma	**0.036**	**0.135**	Old
Vomb	**0.038**	**0.037**	Old

FIGURE LEGENDS

FIG. 1: Map of the study area showing locations of study sites (left), and their position in relation to the rest of Sweden (right). Abbreviations are as follows: F1 = Flyinge 30A1, F3 = Flyinge 30A3, Ge = Genarp, Gu = Gunnesbo, Ha = Habo, Hof = Hofterups, H6 = Höje å 6, H7 = Höje å 7, H14 = Höje å 14, L = Lomma, Va = Vallby, and Vo = Vomb.

FIG 2: Mean F_{st}-values (with SEs) for morph frequencies and neutral data for years 2000 and 2002 for all 12 populations. Data for morph frequencies is based on analysis of allele frequencies estimated using the Hardy-Weinberg formula. Neutral data is based on analysis of 46 putatively neutral AFLP loci. If populations with small sample sizes are excluded, the differences between the types of data become even larger, and differentiation in morph frequencies is significantly higher than expected from drift in the year 2000 (P=0.003), but significantly lower than expected from drift in 2002 (P<0.0001).

FIG 3: Weighted mean Androchrome frequencies with standard errors for 2000 and 2002. There is a significant decrease over time in both the mean Androchrome frequency (P=0.030), and in the variance in Androchrome frequencies (Levene's test: P<0.0001).

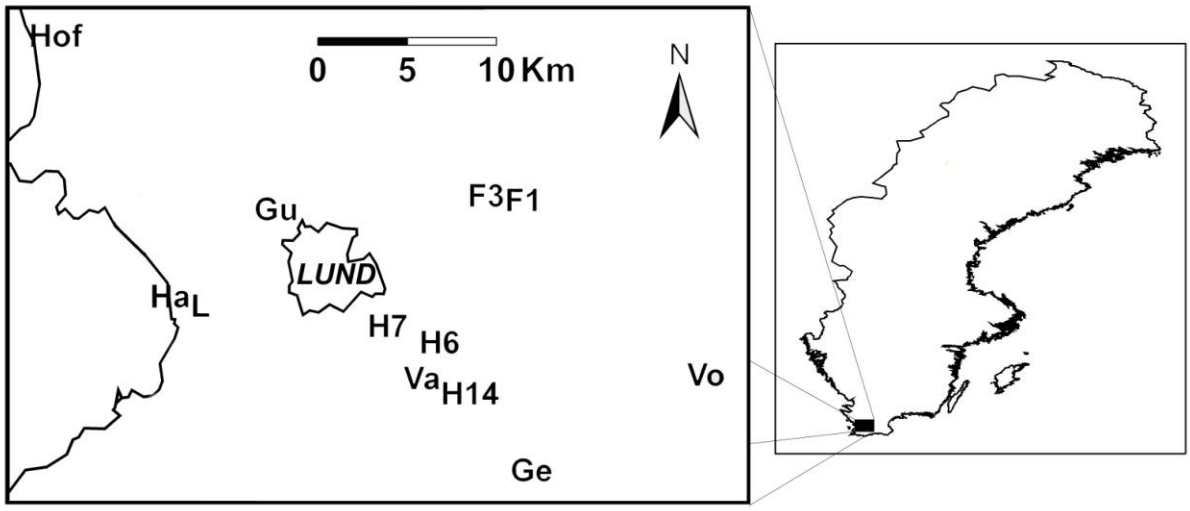

Figure 1

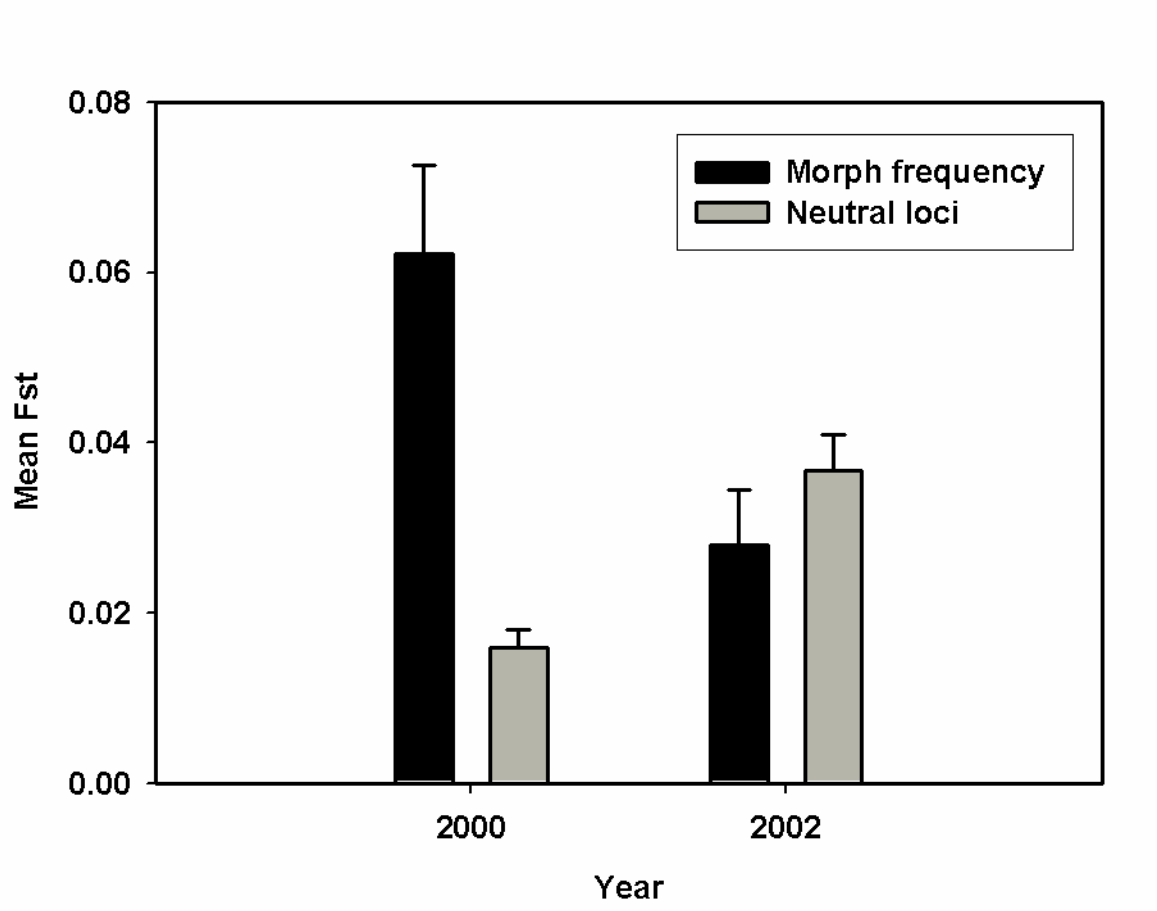
0.08
0.06
0.04
0.02
0.00
Mean Fst
Morph frequency
Neutral loci
2000
2002
Year

Figure 2

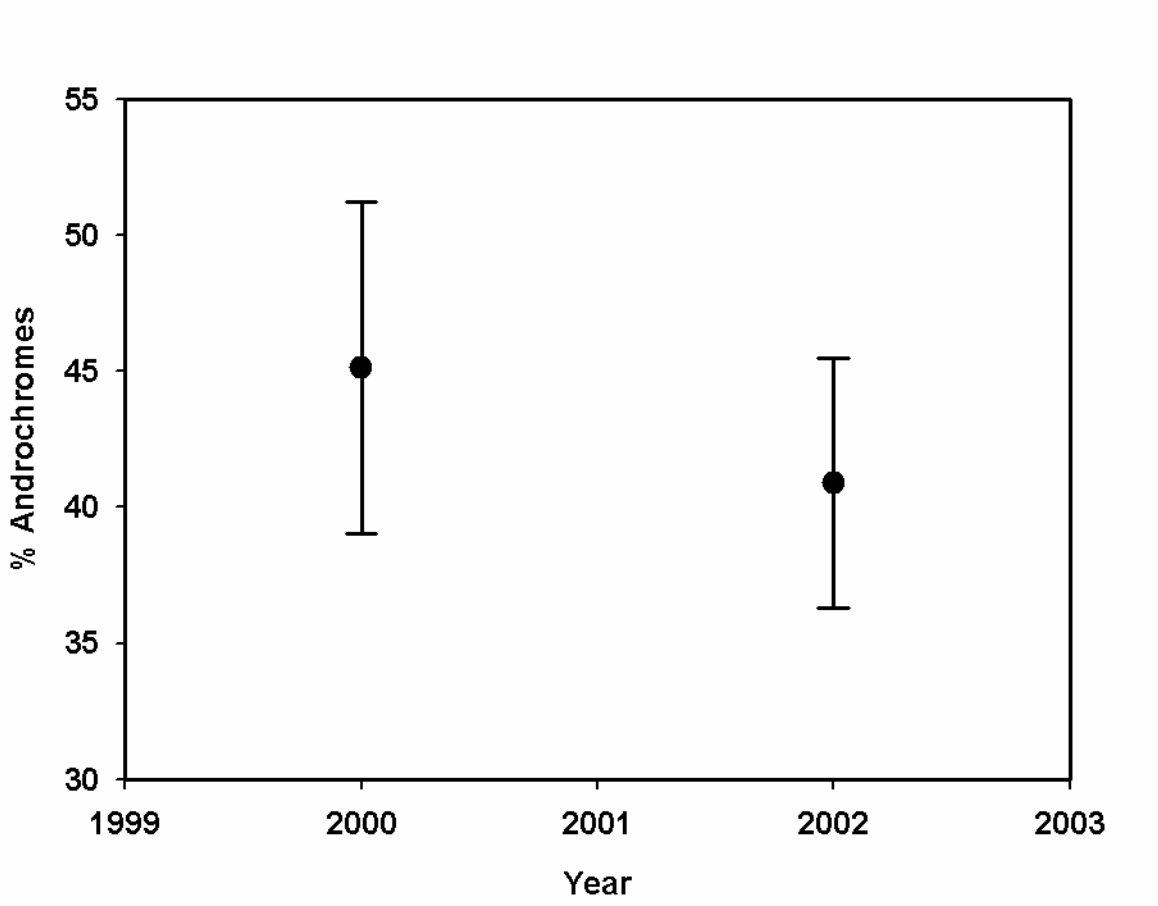

Figure 3