Evolutionary Time-Series Analysis Reveals the Signature of Frequency-Dependent Selection on a Female Mating Polymorphism

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abstract: A major challenge in evolutionary biology is understanding how stochastic and deterministic factors interact and influence macroevolutionary dynamics in natural populations. One classical approach is to record frequency changes of heritable and visible genetic polymorphisms over multiple generations. Here, we combined this approach with a maximum likelihood–based population-genetic model with the aim of understanding and quantifying the evolutionary processes operating on a female mating polymorphism in the blue-tailed damselfly *Ischnura elegans*. Previous studies on this color-polymorphic species have suggested that males form a search image for females, which leads to excessive mating harassment of common female morphs. We analyzed a large temporally and spatially replicated data set of between-generation morph frequency changes in *I. elegans*. Morph frequencies were more stable than expected from genetic drift alone, suggesting the presence of selection toward a stable equilibrium that prevents local loss or fixation of morphs. This can be interpreted as the signature of negative frequency-dependent selection maintaining the phenotypic stasis and genetic diversity in these populations. Our novel analytical approach allows the estimation of the strength of frequency-dependent selection from the morph frequency fluctuations around their inferred long-term equilibria. This approach can be extended and applied to other polymorphic organisms for which time-series data across multiple generations are available.

Keywords: *Ischnura elegans*, negative frequency-dependent selection, balancing selection, color polymorphism, population genetics, maximum likelihood.

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Introduction

While evolutionary genetics has provided powerful analytical and statistical tools for understanding how and why species change across time, understanding how and why species do not change remains a theoretical and empirical challenge. Identifying the potential mechanisms involved in phenotypic stasis remains a central question in evolutionary biology (Futuyma 2010).

Potential explanations for stasis include genetic constraints and stabilizing forms of selection, but both classes of hypotheses face serious difficulties. On the one hand, genetic constraints are difficult to reconcile with the common observation of high levels of genetic variance (Hendry 2013; but see Walsh and Blows 2009). On the other hand, stabilizing selection as an explanation for stasis relies on hypothetical constant ecological factors that stabilize selection (Hansen and Houle 2004). Understanding evolutionary stasis thus requires describing both the underlying causes of genetic variation and the ecological causes of selection. An interesting alternative to stabilizing selection in this context is balancing selection. Indeed, contrary to stabilizing selection, balancing selection—mainly attributed to heterozygote advantage or more general forms of frequency-dependent selection—could explain both evolutionary stasis and high levels of genetic polymorphism in populations.

Organisms with discrete phenotypic polymorphisms, such as color polymorphisms, can aid in understanding both the causes of rapid evolutionary change and observed stasis in morph frequencies, when time-series data are available from several generations (Kettlewell 1961; Sinervo and Lively 1996; Sinervo et al. 2000; Svensson et al. 2005, 2009). The color morphs in the blue-tailed damselfly *Ischnura ele-*

gans provide one example of a stable polymorphism for which such time-series data are available across multiple generations and populations (Gosden et al. 2011). Females of this species can be classified into three nonambiguous adult female color morphs (fig. 1), for which the genetic basis has been well characterized in several multigeneration breeding experiments (Cordero 1990; Sánchez-Guillén et al. 2005). In a continent-wide survey of 120 *I. elegans* populations across Europe, we have previously found that 103 (>85%) contain all three morphs, the reminder being dimorphic with only two morphs (Gosden et al. 2011). This suggests that balancing selection is strong and able to maintain most populations in a trimorphic state, in spite of highly varying microclimatic regimes and local abiotic environments (Gosden et al. 2011). Because the trimorphic system is shared along with the sister species *Ischnura graellsii* (Cordero 1990; Sánchez-Guillén et al. 2005) and polymorphisms are also common in other species of *Ischnura* (Sánchez-Guillén et al. 2011), it is a likely example of an ancient polymorphism that has survived several speciation events. Such female color polymorphisms are common among other coenagrinoid damselflies and odonates in general (Fincke et al. 2005).

Several recent studies have documented observational evidence consistent with negative frequency-dependent selection operating on these damselfly color morphs (Svensson et al. 2005; Gosden and Svensson 2008; Takahashi et al. 2010; Iserbyt et al. 2013). Thus, rare morphs benefit in terms of higher fecundity due to lower male mating harassment (Svensson et al. 2005; Iserbyt et al. 2013). In addition, populations of *I. elegans* with more balanced morph frequencies also have higher per capita female fecundity; they appear to be more stable and suffer from lower extinction risk (Takahashi et al. 2014). One of the three morphs in the *I. elegans* system is considered to be a male mimic because of its male-like coloration and patterning (androchrome females; Cordero 1990; Svensson et al. 2005; fig. 1). These androchrome females are thought to benefit from their intersexual mimicry of males by avoiding excessive mating harassment (Robertson 1985). Negative frequency-dependent selection caused by males harassing the most common female morph is the most likely selective explanation for the maintenance of this polymorphism (Svensson et al. 2005; Gosden and Svensson 2009). Males are thought to form a search image for the most common female morph, as male mate preferences are partly learned and not entirely genetic (Fincke 2004; Svensson and Abbott 2005; Gosden and Svensson 2009; Takahashi et al. 2010; Verzijden et al. 2012; Iserbyt et al. 2013). This mechanism of selection requires that the males' search images switch rapidly, and for this there is some experimental evidence (Van Gossum et al. 2005). A key advantage in using this system is that the genetic basis of the phenotypic trait (female color morph)

is known and the ecological mechanism generating selection is reasonably well understood (see references above). Combined with its discrete univoltine generations, *I. elegans* provide an excellent model system for studying evolutionary change in the wild and for disentangling deterministic (e.g., selection) and stochastic factors (e.g., genetic drift) in time-series data (Svensson et al. 2009).

Although previous work in *I. elegans* and other color-polymorphic damselflies has sought to demonstrate divergent or balancing selection by either combining morph frequency data between populations with molecular markers (Andrés et al. 2000; Abbott et al. 2008; Sánchez-Guillén et al. 2011) or quantifying female fecundities in different density or frequency settings (Svensson and Abbott 2005; Gosden and Svensson 2009; Takahashi et al. 2010; Iserbyt et al. 2013), no study has utilized the quantitative temporal information provided by time series in the form of morph frequency changes across multiple generations and in several populations. In this article, we apply recently developed time-series modeling to infer patterns of frequency-dependent selection in an extensive, multipopulation data set. Our study develops further a population model based on direct fitness estimates in the wild (Svensson et al. 2005) with some recently developed likelihood-based time-series techniques (Le Rouzic et al. 2010, 2011*a*, 2011*b*). Our data and population-genetic model based on the temporal dynamics are consistent with a three-way stable equilibrium maintained by negative frequency-dependent selection. The approach we use here is flexible and can also be tailored to other polymorphic systems for which time-series data are available but where fitness might be difficult to estimate directly. As such, our approach provides an alternative way of inferring the mode and strength of selection, on the basis of observational data in the form of time series. We discuss the scope and limitation of our ability to infer selection from phenotypic time-series data.

Material and Methods

Study Organism, Fieldwork Routines, and Sampling Strategy

Ischnura elegans is a short-lived damselfly with discrete and nonoverlapping generations in northern Europe, where it is univoltine (Svensson and Abbott 2005). The aquatic larval stage lasts about 1 year. Odonates are hemimetabolous insects (Corbet 1999); the larvae undergo an incomplete metamorphosis before entering the adult life stage. In northern Europe, adults start to emerge at the end of May or early June, and the flying season lasts until August (Askew 1988; Abbott and Svensson 2005). Adult males and females go through a series of color changes, whereafter they become sexually mature and mating activity starts (Svensson et al.

Figure 1: Sex-limited color polymorphism in the blue-tailed damselfly (*Ischnura elegans*). *Top*, mating females of the two most common morphs mating with males (males above, female below). *Top left*, *Infuscans* female (I-morph; olive green, no abdominal color patch). *Top right*, androchrome female (A-morph; male-colored mimic, with a blue abdominal patch). *Bottom*, immature *Infuscans-obsoleta* female (O-morph; pink-orange; the blue abdominal patch disappears as the female becomes sexually mature).

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2009). Males are not territorial but engage in scramble competition over females close to the water edge, and females mate with multiple males (Gosden and Svensson 2007, 2009). Copulation lasts for several hours, and during this time both males and females are vulnerable to predation and cannot forage. Precopulatory mating harassment of females by males reduces female fecundity and generates sexual conflict between males and females (Gosden and Svensson 2009; Takahashi et al. 2010).

Females can be easily classified into three discrete color morphs by visual inspection (fig. 1). These three color morphs are only expressed at the adult life stage and are not visible during the larval part of the life cycle. Females belonging to the *Infuscans* morph (I-morph) are olive green and lack a blue abdominal color patch. Androchrome females (A-morph) are male mimic (blue), with a blue abdominal patch. The rarest morph is *Infuscans obsoleta* (O-morph), characterized by an orange-pink color. All three female morphs occur in the 12 populations of this study, albeit at different frequencies.

The data used in this study come from a long-term population study on *I. elegans* that has been ongoing since the reproductive season of year 2000. The entire study covers the period up until 2011, a total of 12 seasons (i.e., 12 generations) from 12 intensively monitored populations in a small geographic area (ca. 40 × 40 km²) outside Lund, Province of Skåne, southern Sweden (Gosden and Svensson 2008; Gosden et al. 2011). These populations are the same as those used in a previous study, monitoring frequency changes over the first four generations (Svensson and Abbott 2005; Svensson et al. 2005), except that the population Fjelie became extinct early in the study period and is not included here. Additional information about geographic locations and general ecological information about these populations can be found elsewhere (Svensson and Abbott 2005; Abbott et al. 2008). Most populations were sampled three to four times each summer with weekly intervals, except when cold and rainy weather prevented fieldwork. At each visit, we recorded our catching effort (in minutes) as we tried to catch as many males and females as possible, both those flying alone (single) and those found mating (couples).

After capture, individuals were transported to an indoor laboratory at the Biology Department in Lund, where they were sorted and classified with respect to sex, mating status (found copulating or single) and female color morph (A-morph, I-morph, or O-morph). From these field capture data, we calculated the total number of individuals caught and the total numbers of males and females. From this, we computed female morph frequencies, adult sex ratio, and indices of male and female densities (number of individuals caught per minute). The total sample size in this study across all 12 seasons (2000–2011) and all 12 popula-

tions amounts to 6,413 females. Female morph frequencies were available for most populations in most years, except in a few cases where population densities were too low or no animals were caught, in spite of several visits. Frequencies were estimated in all years as well as densities (except in the first season, where we did not record our catching effort).

Population-Genetics Model

Frequency-dependent selection was modeled within an explicit population-genetics framework. The genetic basis of the color morphs is a triallelic system with specific dominance relationships. This genetic architecture is identical in *I. elegans* and its sister species *Ischnura graellsii*, as experimentally documented in two previous multigeneration breeding experiments in common laboratory environments (Cordero 1990; Sánchez-Guillén et al. 2005).

The expression of the three morphs A, I, and O is caused by three underlying alleles (α, β, and γ) at a single autosomal locus (Cordero 1990). Genotypes $\alpha\alpha$, $\alpha\beta$, and $\alpha\gamma$ lead to morph A, genotypes $\beta\beta$ and $\beta\gamma$ to morph I, and genotype $\gamma\gamma$ to morph O. Thus, the three alleles follow the hierarchical dominance pattern $\alpha > \beta > \gamma$. The frequencies of the three morphs P_A, P_I, and P_O are deduced from the three allele frequencies denoted p, q, and r.

There are solid theoretical arguments to consider Wrightian fitness on a multiplicative scale (Wagner 2010). Frequency-dependent selection is thus modeled by a log-fitness function changing linearly with morph frequencies (the fitness of morph O being taken arbitrarily as a reference):

$$\log(W_A) = s(P_A - P_A^*),$$
$$\log(W_I) = s(P_I - P_I^*), \tag{1}$$
$$\log(W_O) = 0.$$

The selection model thus relies on three independent parameters: two equilibrium frequencies (P_A^* and P_I^*) and a frequency-dependent selection coefficient (s). The equilibrium frequency of morph O can be easily deduced from the estimated equilibrium parameters, $P_O^* = 1 - P_A^* - P_I^*$. The parameter s can be positive or negative, leading to either positive or negative frequency-dependent selection, respectively.

Assuming that male genotype does not interact with female choice (i.e., mating is random after selection), morph frequencies after reproduction and before selection are

$$P_A = p^2 + 2pq + 2pr,$$
$$P_I = q^2 + 2qr, \tag{2}$$
$$P_O = r^2.$$

Allele frequencies after selection are derived as

$$p' = \frac{pW_\mathrm{A}}{\overline{W}},$$

$$q' = \frac{q[pW_\mathrm{A} + (1-p)W_\mathrm{I}]}{\overline{W}}, \tag{3}$$

$$r' = 1 - p' - q',$$

with $\overline{W} = P_\mathrm{A}W_\mathrm{A} + P_\mathrm{I}W_\mathrm{I} + P_\mathrm{O}$.

Statistical Model

The purpose of the statistical approach in this article is to estimate the parameters underlying the population-genetic model (the selection coefficient s, assumed to be the same across the whole system, and population-specific morph equilibrium frequencies, $P_{\mathrm{A}_i}^*$ and $P_{\mathrm{I}_i}^*$) from the empirical data set. The data consist of the numbers of females for each morph ($N_{\mathrm{A}_{t,i}}$, $N_{\mathrm{I}_{t,i}}$, and $N_{\mathrm{O}_{t,i}}$) in every population i and for each year t.

The statistical framework includes two sources of stochasticity: (1) sampling effects, representing the uncertainties about the measured frequencies in the populations; and (2) true genetic drift, which disturbs the morph frequencies, pushing them away from potential equilibrium points and generating selective forces that can be caught by the model. This setting is not specific to frequency-dependent selection, and equivalent models are already used in evolutionary ecology, for example, to estimate density dependence (Dennis and Taper 1994; Dennis et al. 2006).

Sampling effects are modeled in the following way: the probability of sampling N_A, N_I, and N_O individuals in a given population at a given generation follows a multinomial distribution $\mathrm{Prob}_t \sim \mathcal{M}(N_\mathrm{A}, N_\mathrm{I}, N_\mathrm{O}|P_\mathrm{A}, P_\mathrm{I}, P_\mathrm{O})$, where P_A, P_I, and P_O are the theoretical frequencies predicted by the model. Prob_t thus quantifies the probability of observing the data (N_A, N_I, and N_O in each population for every year of the time series), given the theoretical prediction of the model.

Genetic drift also follows a multinomial sampling process: the probability of having allele frequencies (p_{t+1}, q_{t+1}, r_{t+1}) at generation $t + 1$ follows the multinomial distribution $\mathrm{Prob}_{t \to t+1} \sim \mathcal{M}(2N_e p_{t+1}, 2N_e q_{t+1}, 2N_e r_{t+1}|p_t', q_t', r_t')$, where primed variables indicate allele frequencies after selection.

The parameters of interest (s, P_A^*, and P_I^*) cannot be estimated without considering allele frequencies at every generation. The likelihood of the parameters for a time series (generations 1 to T) in one population can be written as

$$L(s, P_\mathrm{A}^*, P_\mathrm{I}^*, p_1, \ldots p_T, q_1, \ldots, q_T)$$

$$= \mathrm{Prob}(N_{\mathrm{A}_1}, \ldots, N_{\mathrm{A}_T}, N_{\mathrm{I}_1}, \ldots, N_{\mathrm{I}_T}, N_{\mathrm{O}_1}, \ldots, N_{\mathrm{O}_T}) \tag{4}$$

$$= \prod_{t=1}^{T} \mathrm{Prob}_t \times \mathrm{Prob}_{t \to t+1}.$$

The combined likelihood function for the whole system was obtained by multiplying the likelihoods for the individual populations. This assumes that the dynamics of the different populations are independent. Parameter estimates were obtained by numerically maximizing the combined likelihood function. Each population has its own equilibrium frequencies (two parameters for each population), but the strength of selection, s, is shared across the whole system. We detail the challenges of relaxing this assumption in "Discussion." In sum, fitting the model requires estimating a single selection coefficient s, 24 equilibrium morph frequencies ($P_{\mathrm{A}_i}^*$ and $P_{\mathrm{I}_i}^*$ for each population i), and 288 allelic frequencies (144 $p_{i,t}$ and 144 $q_{i,t}$ for each of the 12 populations i at each of the 12 generations t).

For numerical reasons, we calculated multinomial probabilities with a continuous approximation ($x! = \Gamma(x + 1)$), because model-fitting procedures require allele frequencies to vary continuously. Moreover, the model was fit for $\ell_{p_t} = \log(p_t/r_t)$ and $\ell_{q_t} = \log(q_t/r_t)$, and frequencies were back-transformed after convergence. The effective population size was considered as constant and fixed to $N_e = 50$. The consequences of changing this value are minor, as shown in figure 2B. The maximization was performed with the software ADMB (ver. 11; Fournier et al. 2012), which is designed for solving high-dimensional, nonlinear problems. According to equation (4), the likelihood function was coded (in the C++ dialect used by ADMB) as the product of two multinomial probabilities across generations and populations. The 288 allelic frequencies are not of direct interest and could be treated as nuisance parameters in a random-effect setting. Unfortunately, numerical convergence appeared to be particularly tricky when integrating out these parameters in the likelihood function, probably because of a strong correlation structure between random effects. On the contrary, convergence was not problematic when all parameters were treated as fixed effects, provided that reasonable starting values were used. Additional simulations (not shown) were performed to verify that the statistical properties of parameter estimates were not substantially different in fixed versus random effect settings.

Among other indicators, the software reports maximum-likelihood estimates of parameters as well as their variance-covariance matrix (based on the Hessian matrix), which was used to derive standard errors and approximate confidence intervals. Numerical simulations and data analysis were performed in R (ver. 3.0; R Core Team 2013). The
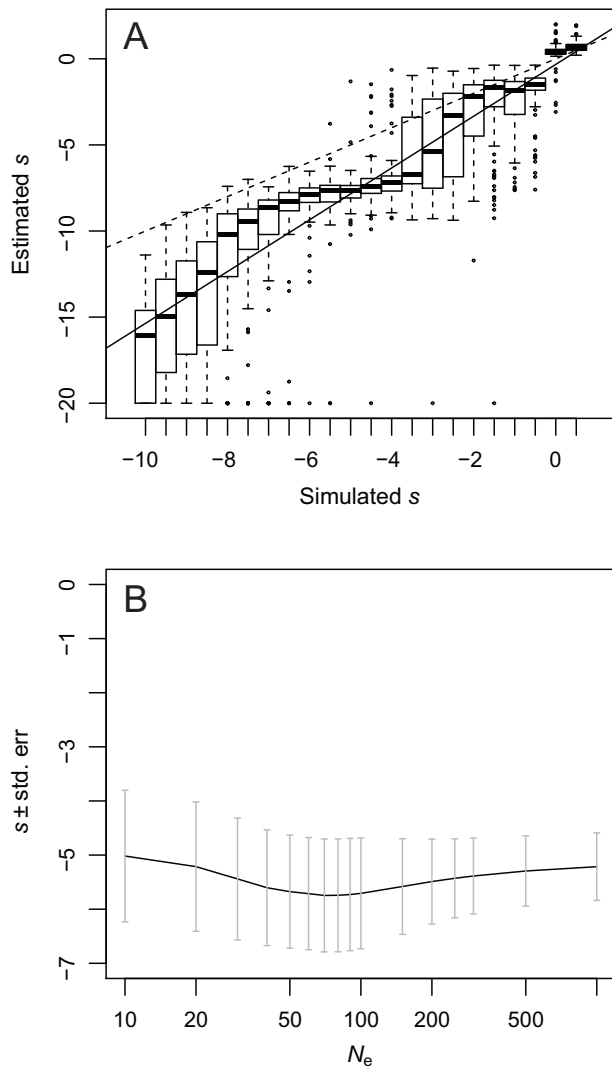
Model Testing

Bias in the Selection Parameter. The model is nonlinear, and maximum-likelihood estimates are often affected by various biases in similar settings. Biased estimates are not necessarily problematic, especially when the bias can be quantified, which makes it possible to correct the estimate. A hundred individual-based simulations were run for a series of selection coefficients distributed between 1 and −10 with a setting matching the data (12 populations with a sample size drawn into an exponential distribution of an average of 44.5 individuals per year and per population). These simulations reproduced the population-genetic model described above, with equilibrium frequencies equal to the mean frequencies observed in the data. In addition, sample sizes around 10 times greater (400 individuals per population and per year) were also simulated to evaluate the statistical properties of larger data sets.

Alternative Settings. In order to challenge this basic model and test for different settings, we defined and implemented alternative models in the same software environment. In particular, we assessed the influence of the selection model by allowing alternative parameterization of the fitness functions of equation (1). First, a more complex model (hereafter, the two-parameter selection model) was defined with two independent selection parameters ($\log(W_A) = s_A(P_A − P_A^*)$, $\log(W_I) = s_I(P_I − P_I^*)$, and $\log(W_O) = 0$). Second, various combinations based on the original model were generated in which one (three models), two (three models, including the one of eq. [1]), and three (one model) morphs are affected by their own frequency.

Goodness-of-Fit. Model selection picks the model that best fits the data among a predefined set of models, but it cannot evaluate how good the model is at fitting the data. We performed a goodness-of-fit test based on a Monte Carlo method (Waller et al. 2003): 1,000 simulations parameterized with the maximum-likelihood estimates of selection and frequency parameters were run for 12 populations, with the same sample sizes as in the real data. These simulations were analyzed with the statistical model, and the distribution of the likelihood values was used to check to what extent the data constitute a typical realization of the model.

Figure 2: Model validation. *Top*, simulated versus estimated values of *s* (500 simulations for each value of $−10 < s < 1$) in the small population setup ($N = 44.5$). Boxes represent the quartiles of the distributions, with outliers ($>1.5 ×$ the interquartile range) being displayed as circles outside boxes. The dashed line shows the expected pattern (estimated = simulated), and the solid line is an ordinary regression (estimated = $1.45 ×$ simulated − 0.44). *Bottom*, effect of changing the arbitrary value of N_e on the selection coefficient estimates. The model was fit to the real data for a range of N_e values; the figure reports the maximum-likelihood estimate and the associated standard error.

data set (morph frequencies and sample sizes in the different populations and years) as well as the computer code (R scripts) behind the figures and results in this study are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.bj86g (Le Rouzic et al. 2015).

Results

Model Validation

Before fitting our model to the empirical data, we evaluated the accuracy of the statistical procedure, using simulated data for which the selection strength was known. We investigated (1) whether the model itself and its implementation were able to estimate parameters of interest (basically,

we checked whether an estimate of frequency-dependent selection based on stable time series could be obtained) and (2) whether the size of the current data set was sufficient to measure model parameters with reasonable precision. Two models were tested, as described in "Material and Methods": the one-parameter model of equation (1) and a two-parameter model with different strengths of selection s_A and s_I for both morphs. This two-parameter model was functional but gave only reasonably precise estimates for the larger (400 individuals) simulations. Given that our empirical sample size was much lower than this (average of 44.5 individuals per population), we deemed it unrealistic to independently estimate the selection coefficients for both morphs; we therefore focused on the single-parameter model, assuming the same relative selection strength on the different morphs.

Simulations showed that this single-parameter model was able to estimate the average selection coefficient (fig. 2A). However, when the sample size is the same as in the data (44.5 females per year and per population on average), the selection estimate is biased. On the basis of the simulation results, the parameter s tends to be overestimated. These simulations confirm that the model is unlikely to generate false positives (simulations with no selection lead to estimates very close to $s = 0$). The bias vanishes for larger sample sizes (not shown), indicating that it is not due to an implementation bug.

We also investigated the sensitivity of the model to the effective population size N_e, which conditions the strength of genetic drift and has to appear as a given parameter in the model. Running the model on the data set with several values of N_e ranging from 10 to 1,000 showed virtually no effects on the estimated value of s, the major impact being the narrowing of the confidence interval around s for large N_e (fig. 2B). The selection coefficient cannot be estimated for very large values of N_e (the model does not converge), since genetic drift is necessary to disturb the equilibrium. Using an arbitrary value of $N_e = 50$, roughly estimated from the observed number of individuals in our experimental ponds, is unlikely to affect the output of the model.

A thousand Monte Carlo simulations based on the estimates obtained from the real data set showed that only six out of 1,000 simulations fit the real data better than the simulated data. This lack of fit can be attributed to the simplicity of the model, but we could not achieve a significantly better fit by combining the selection coefficients in equation (1) differently (see below).

Estimation of the Mode and Strength of Selection

The model was fit to the full data (2000–2011, 12 populations and 12 generations; fig. 3). The maximum-likelihood estimate for the frequency dependence parameter is (estimate $\pm$ SE) $s = -5.67 \pm 1.05$, which makes frequency dependence statistically significant (H_0: no frequency dependence [$s = 0$], $P < 10^{-7}$ if the error is assumed to be Gaussian). The sign of the estimated coefficient is negative, meaning that a morph's fitness is expected to decrease as its frequency increases (i.e., negative frequency-dependent selection). Correcting the estimate for statistical bias illustrated in figure 2A leads to $s_{corr} = s/1.45 = -3.91$ for $s = -5.67$, which does not change our main qualitative conclusions. The coefficient s can be interpreted as the change in fitness generated by a (small) change in frequency around the equilibrium: $s = -5.7$ means that a 1% shift in morph frequency away from equilibrium generates a 5.7% (raw) or 3.9% (bias-corrected) change in fitness. Figures 4 and 5 represent the fitness functions in morph frequency space, assuming a population with average morph equilibrium frequencies.

By construction, frequency dependence is symmetric: selection on two of the morphs necessarily induces frequency-dependent selection of the same sign on the third morph. Here, we chose to model selection explicitly on the two most frequent morphs, A and I. Alternative models in which frequency dependence was applied on different morphs (A and O, I and O, as well as on all three morphs simultaneously) gave similar values for s (e.g., $s = -5.81$ when all three morphs are affected by frequency dependence on the absolute fitness). The likelihood values were also quite similar with less than three Akaike information criterion (AIC) units difference between models, showing that all these models have roughly similar explanatory powers. Thus, the choice of a specific model is unlikely to affect the conclusions. In contrast, applying selection on morph A alone led to a difference of about six AIC units, suggesting that frequency-dependent selection on morph A cannot fully explain the stability observed in this trimorphic system. In practice, this also means that the frequencies of the two less common morphs are too stable across generations to be explained by genetic drift alone.

Morph Equilibrium Frequencies

Estimated equilibrium frequencies varied between 0.56 and 0.80 across populations for morph A, between 0.04 and 0.26 for morph I, and between 0 and 0.23 for morph O (fig. 6). Differences among populations are too large to be due to estimation errors, since the confidence ellipses do not overlap. We attempted to correlate equilibrium-frequency differences with available population-specific measurements, including population density (number of individuals caught per minute), sex ratio, geographical location, and pond size (area and perimeter). None of these factors were convincingly associated with morph equilibrium frequencies. In five out of 12 populations, the equilib-
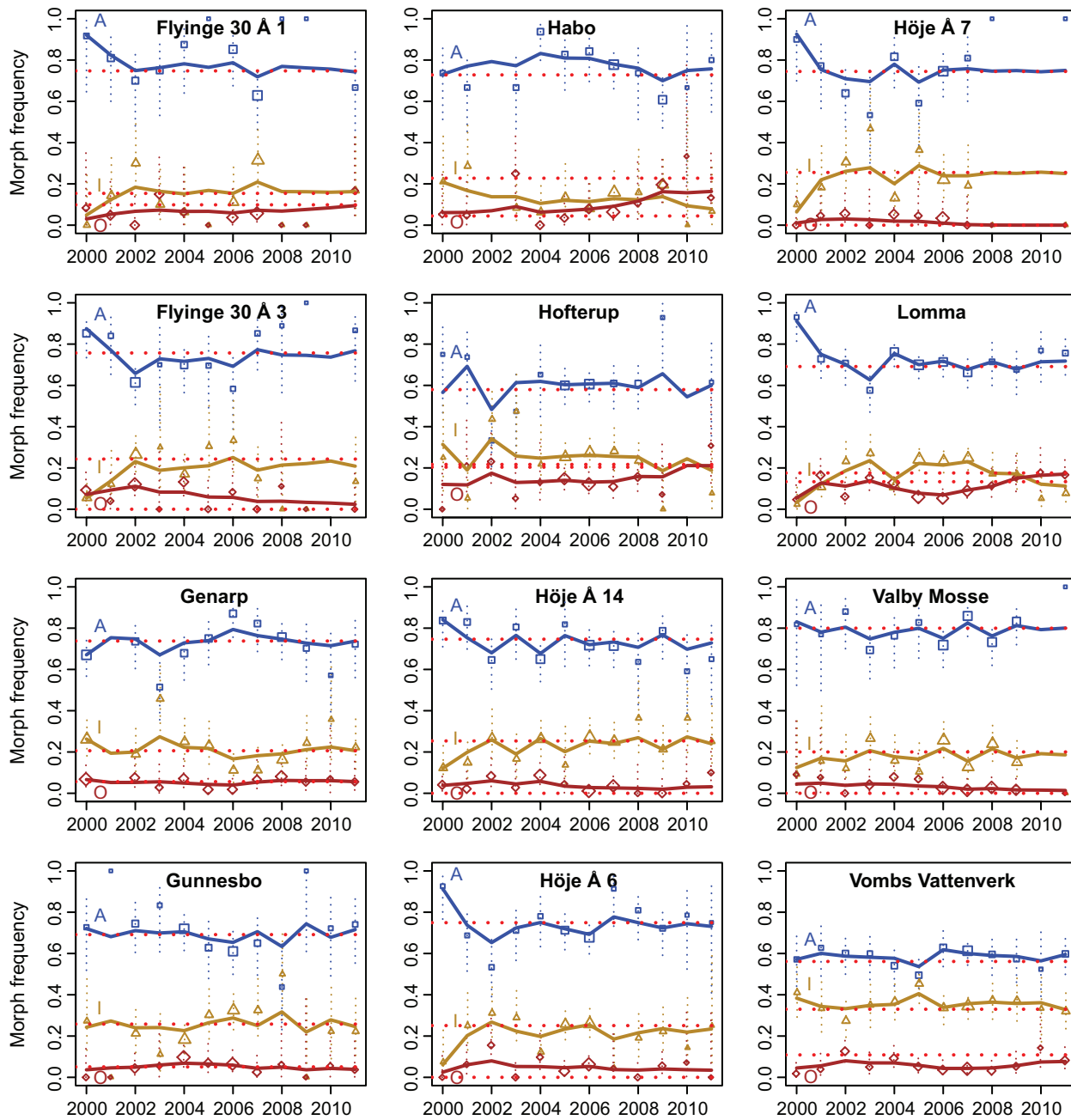
Figure 3: Observed (points) and estimated (lines) morph frequencies for each of the 12 populations. Blue, A morph; beige, I morph; brown, O morph. The size of the points is proportional to the sample size, and dotted segments stand for approximate 95% confidence intervals (not computed for $N < 3$ to avoid overcrowding figure). Estimated equilibrium frequencies are indicated as horizontal dotted lines.

rium frequency of morph O was too small to be estimated properly.

Three populations appear as outliers. In population Habo, the frequency of the I-morph tends to decrease with time, thus explaining the low predicted equilibrium frequency and suggesting that the population might not be

at equilibrium. On the opposite end of the scale, two populations, Vombs Vattenverk and Hofterup, have unusually low A-morph frequencies and do not display any obvious signs of nonequilibrium. At present, we have no convincing explanation for these differences between closely located populations, although we note that they are also genetically
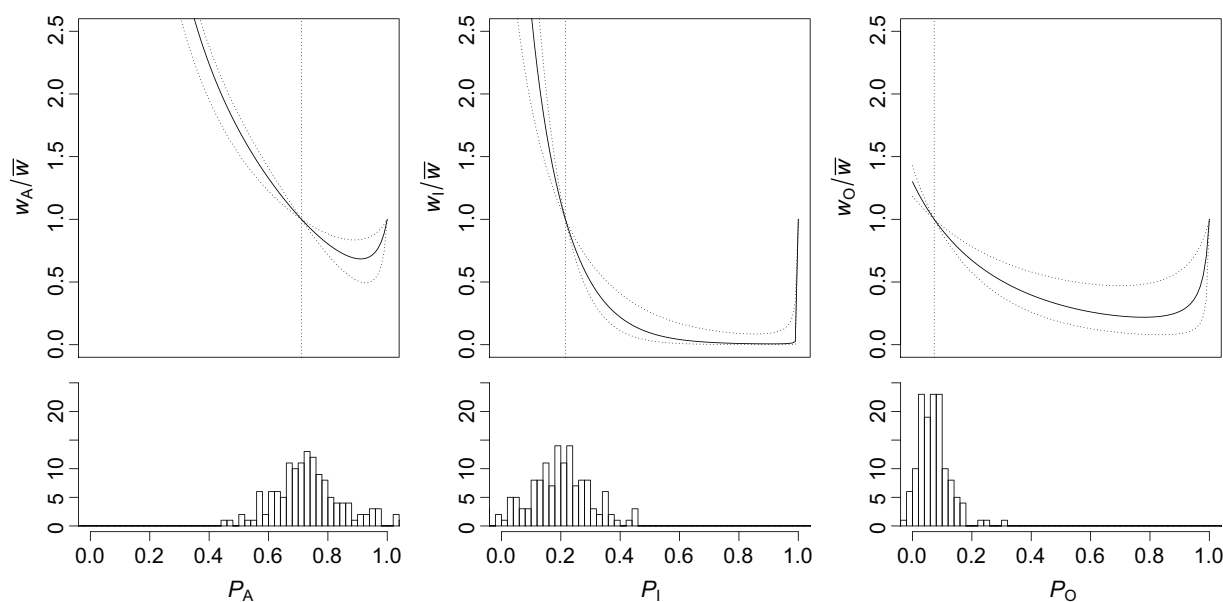
Figure 4: Relative fitness of the three morphs as a function of morph frequency. The solid line depicts the estimated fitness landscape, and the dotted lines stand for approximate 95% confidence intervals. The calculation assumes that the nonfocal morph frequencies are at the same ratio as their mean values ($\bar{P}_A = 0.71$, $\bar{P}_I = 0.22$, $\bar{P}_O = 0.07$). Vertical bars indicate equilibrium frequencies for which all morph fitnesses are identical. The fitness functions all have negative slopes in relation to morph frequency, illustrating negative frequency-dependent selection. The bottom panels display the distribution of frequencies in the data set (12 years × 12 populations = 144 frequencies). Frequencies were corrected for population-specific effects; that is, the population mean was removed and replaced by the grand mean, explaining why frequencies can occasionally span beyond the [0, 1] interval.

differentiated with respect to neutral (amplified fragment length polymorphism) markers (Abbott et al. 2008).

Discussion

Estimating Selection

Natural selection is at the foundation of evolutionary thinking, but during the modern synthesis, it was studied more from a theoretical than from an empirical point of view (Provine 1971). The situation changed in the early eighties with the development of selection gradient analysis, as outlined in the seminal article by Lande and Arnold (1983). This work and subsequent extensions brought powerful statistical tools that evolutionary biologists could use to measure directional selection in the wild, which spurred a wave of studies of natural and sexual selection (reviewed in Endler 1986; Kingsolver et al. 2001; Hereford et al. 2004; Stinchcombe et al. 2008; Siepielski et al. 2009, 2011, 2013; Calsbeek et al. 2012; Morrissey and Hadfield 2012).

There are currently a multitude of studies that have not only documented the operation of selection in the field but also sought to estimate its strength and mode. Despite the apparent success of this approach, the empirical analysis of selection suffers from a number of methodological shortcomings stemming from both statistical and measurement-

theoretical issues, which hamper our ability to interpret and generalize findings across studies. In addition, it has been pointed out that it is not enough to identify the target traits of selection but also the ecological causes and selective agents (Sober 1984; Wade and Kalisz 1990).

The past few years have seen active research aimed at addressing some of the shortcomings of the classic linear regression approach with specific improvements in the techniques used for estimating phenotypic selection in a variety of data types. This includes more flexible parametric models of the adaptive landscape (e.g., Schluter 1988; Schluter and Nychka 1994; Blows and Brooks 2003; Blows 2007; Bolstad et al. 2010; Shaw and Geyer 2010; Chenoweth et al. 2012; Pélabon et al. 2012; Morrissey and Sakrejda 2013), models incorporating frequency- or density-dependent selection (Álvarez-Castro and Álvarez 2005; O'Hara 2005), a better awareness of the assumptions and consequences of scaling (Hereford et al. 2004; Stinchcombe 2005; Stinchcombe et al. 2008; Wagner 2010; Hansen et al. 2011; Houle et al. 2011; Matsumura et al. 2012), consideration of measurement error (Hereford et al. 2004; Morrissey and Hadfield 2012), methods for studying selection on the genetic component of traits (Stinchcombe et al. 2002; Winn 2004; Reid and Sardell 2012; Morrissey 2014), techniques for causal and hierarchical decomposition of selection
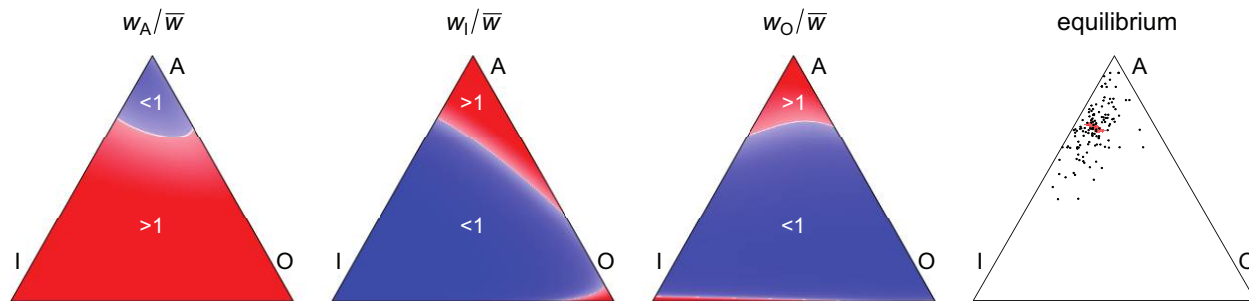
Figure 5: Multidimensional fitness functions of the morphs illustrated using ternary plots. Equilibrium frequencies are taken as the mean over all populations. The triangular plots represent the fitness surfaces in morph frequency space for each of the three morphs. Red, relative morph fitnesses >1; blue, relative morph fitnesses <1. Note that all three morphs are predicted to have relative fitness <1 (blue) as they approach 100% in frequency, consistent with negative frequency-dependent selection and a disadvantage of common morphs. The frequency of, for example, morph A is 100% at the corner A and decreases linearly toward the opposite edge of the triangle. In the last triangle, the red area indicates the frequency space in which the system is approximately at equilibrium (all three relative fitnesses between 0.9 and 1.1) as well as the range of observed frequencies (corrected for the population mean).

(Heisler and Damuth 1987; Armbruster 1991; Scheiner et al. 2000; Ridenhour 2005; Walker 2007; Chenoweth et al. 2012), and models to infer selection from evolutionary time series (Édeline et al. 2009; Le Rouzic et al. 2010, 2011*a*).

We have proposed and developed a methodological framework aimed at measuring the strength of balancing selection based on the statistical analysis of an empirically quantified time series. The task is challenging: contrary to directional selection, which generates trends in allele frequencies or phenotypic means that can be used to estimate selection strength (Le Rouzic et al. 2011*a*, 2011*b*), negative frequency-dependent selection at equilibrium must be detected through the behavior of the system in response to stochastic perturbances. Negative frequency-dependent selection may act as a conservative force that limits population divergence (Svensson et al. 2005), and it erases its own traces in a similar fashion to stabilizing selection. Morph frequencies are indeed remarkably stable over the study period (a decade) and across our 12 study populations (fig. 3). We interpret this as selection generating a strong force that punishes strong deviations from the long-term morph frequency equilibria (figs. 3, 6). Note that we cannot formally exclude other evolutionary mechanisms that would result in similar patterns of frequency time series. In particular, long-term stability can also be maintained by a significant influx of recurrent migrations from neighboring patches. However, migration is expected to homogenize allele frequencies across populations and cannot explain different allele frequencies across populations. There are also no clear geographical patterns or spatial autocorrelation in allele frequencies. This suggests local differences in the selection regime as the cause of the differences.

Here, the modeling task was complicated by the trimorphic state of the populations. In a dimorphic system, only one frequency-dependent selection coefficient is necessary to fully describe the equilibrium properties of the population, whereas six parameters (including the influence of the ratio of two morphs on the fitness of the third one) are necessary for an exhaustive mathematical description of a trimorphic system. Moreover, intuitive reasoning is difficult because of various symmetries in the model. For instance, the model described in equation (1) includes frequency dependence for morph O, given that any frequency change in O necessarily generates a change in the relative fitness of O as a result of induced changes in fitnesses of A and I. This six-parameter model has interesting mathematical properties and allows for unstable or cyclic equilibria. Unfortunately, fitting such complex models in our data sets is out of reach. The one-parameter model of equation (1) may seem arbitrary, but alternative models of similar complexity did not display additional explanatory power, including (1) various permutations between morphs around the pattern of equation (1), (2) linear (vs. log-linear) fitness functions, and (3) a random effect setting allowing *s* coefficients to be normally distributed across populations (the estimated variance for *s* being estimated to 0). Therefore, even if the goodness-of-fit test shows that the model does not fit the data perfectly, it is unlikely that this can be attributed to the fitness function.

Our population-genetics model is based on prior information about the natural history, ecology, and the genetic basis of the female polymorphism in *Ischnura elegans*. This approach enabled us to separate the stochastic effects of genetic drift from those of sampling and to identify the underlying deterministic effects of selection. However, the simplicity of distinct morphotypes with a one-locus Mendelian basis is not characteristic of all study systems. Nevertheless, our approach is flexible and can be implemented
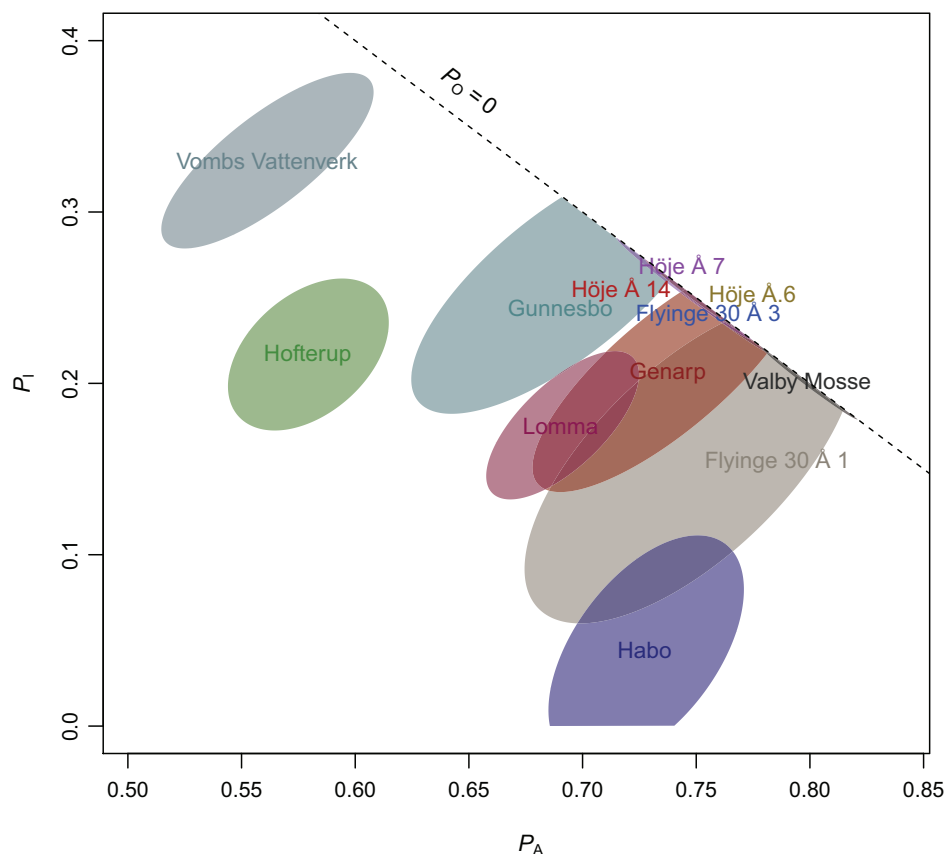
Figure 6: Position of the estimated equilibrium frequencies in the morph frequency space for all 12 populations. Ellipses illustrate the standard errors of equilibrium frequencies, derived from the variance-covariance matrix of the estimates. In five populations, the estimated equilibrium for the O morph cannot be statistically distinguished from 0.

and extended to other polymorphic systems for which time-series data are available or can be collected, such as pollination polymorphisms (e.g., Eckert and Barrett 1995; Ågren and Ericson 1996; Barrett 1998); color polymorphisms (e.g., Kettlewell 1961; Sinervo and Lively 1996; Sinervo et al. 2000; Svensson et al. 2009); male and female mating polymorphisms in fish, insects, birds, and several other taxa (e.g., Neff and Svensson 2013; Karlsson et al. 2014); and, of course, any other Mendelian traits. In particular, our approach is agnostic regarding the mechanisms underlying frequency-dependent selection, since it makes only two main assumptions: (1) there is a set of morph frequencies for which fitnesses are identical (equilibrium), and (2) fitness changes exponentially with morph frequency. This allows the equilibrium frequencies and the strength of selection to be estimated independently of each other, which is not the case when fitness functions are defined relative to the morph frequency alone (in which case the equilibrium depends on a complex combination of frequency-dependent parameters for each morph).

Frequency-Dependent Selection in Ischnura elegans *and Other Damselflies*

Although previous studies on color-polymorphic damselflies have estimated selection coefficients more directly with fecundity data of females from different populations with different morph frequencies (Svensson and Abbott 2005; Takahashi et al. 2010), there has been no attempt to evaluate the importance of negative frequency-dependent selection and estimate its strength from a time series spanning multiple generations and populations. Such indirect approaches have some advantages over the more direct approach of estimating fecundities in the field. First, fecundity differences between female morphs will reflect selection only at the adult stage and hence are only one component of fitness, whereas selection coefficients estimated from time-series data—such as the ones we present here—will reflect total selection on the morphs integrated over the entire life cycle, encompassing both larval and adult stages. Second, by estimating selection coefficients from time se-

ries data, we confirm that balancing selection is a consistent explanation for both the observed fluctuations in morph frequencies and their remarkable stability, spanning more than a decade of field observations. Our modeling results and the estimated selection coefficient provide an independent line of evidence for frequency-dependent selection in addition to our previous experimental and observational studies (Svensson et al. 2005; Gosden and Svensson 2009).

We note that neither this study nor our previous studies in which we estimated selection coefficients associated with different morph frequencies (Svensson et al. 2005) can, in themselves, demonstrate the actual ecological mechanism generating the frequency-dependent selection. However, there is additional experimental and behavioral evidence that the mechanism generating frequency-dependent selection in this system is indeed caused by fitness costs associated with increasing mating harassment as a given morph density increases in the population (Gosden and Svensson 2009). Therefore, we suggest—on the basis of the time-series analysis results in this study in combination with previous experimental and behavioral studies on this and closely related damselfly species (Takahashi et al. 2010; Iserbyt et al. 2013)—that the inferred decline in the fitness of female morphs (figs. 4, 5) are indeed caused by apostatic selection due to mating harassment as any given morph becomes more common in the population.

More generally, mating harassment and sexual conflict over mating rates were at first thought to promote population divergence and facilitate speciation (Gavrilets 2000), but it has since been recognized that such sexual conflict could also promote female mating polymorphisms within populations and lead to the emergence of distinct sympatric female genetic clusters (Gavrilets and Waxman 2002; Svensson and Abbott 2005; Svensson et al. 2009; Karlsson et al. 2013, 2014). In our case, apostatic sexual conflict leads to multiple female morphs both locally and regionally, and it opposes the stochastic loss of morphs through genetic drift in local populations. Hence, frequency-dependent sexual conflict can play a conservative role, preventing population divergence in morph frequencies and hindering the stochastic transition of local polymorphic populations into monomorphic ones (Svensson and Abbott 2005; Svensson et al. 2005).

Our estimated selection coefficients show that frequency-dependent selection is a strong enough force in this system to override genetic drift in local populations, which has been implicated to interact with selection in some other polymorphic systems (Runemark et al. 2010). This conclusion is reinforced by our findings from a large-scale biogeographic study, where we found that the vast majority of all populations of *I. elegans* contain all three morphs, with only a small fraction of dimorphic and no monomor-

phic populations (Gosden et al. 2011). Yet in southern Europe, the A-morph is typically around 30% (Gosden et al. 2011), which is radically different from our study area in Sweden, where the A-morph is more common, typically between 60% and 80% (Svensson and Abbott 2005). Future time-series data on morph-frequency fluctuations from southern populations could perhaps generate the statistical power to address additional questions, such as whether the selection coefficient *s* differs in magnitude between the different morphs and/or populations.

Concluding Remarks

Many different ecological agents of selection have been identified during the past century, and some progress has been made in estimating natural selection in the wild at the microevolutionary level. Yet there is still plenty of work needed to help in understanding and predicting how species evolve on microevolutionary timescales, partly because of our inability to disentangle the respective impact of various selection forces and stochastic factors on populations. Quantifying the strength of frequency-dependent selection from observations on unmanipulated populations in the wild is a challenging task, but studies of this nature can help to shed light on the processes leading to either stasis or change over macroevolutionary timescales. The model we proposed here demonstrates that it is both theoretically and empirically possible to estimate the strength of frequency-dependent selection from data on the genetic dynamics of equilibrium populations. Yet this was possible only because of the exceptional quantity and quality of the data set (more than 6,000 phenotyped individuals collected over a 12-year period) in a study system in which the genetic basis of the trait and the causes of selection, mating system, natural history, and ecology of the species are reasonably well understood. This study has revealed that the remarkable stability of the morph frequencies over 10 generations is consistent across multiple populations and reveals stasis at microevolutionary timescales that is best explained by negative frequency-dependent selection. Generalizing the approach we have used to a wider range of traits and species may be an important step toward a better understanding of the stability or dynamics of adaptive landscapes and the selective processes leading to either rapid evolutionary change or stable evolutionary equilibria (Arnold et al. 2001; Estes and Arnold 2007; Calsbeek et al. 2012; Hansen 2012; Svensson and Calsbeek 2012; Arnold 2014).

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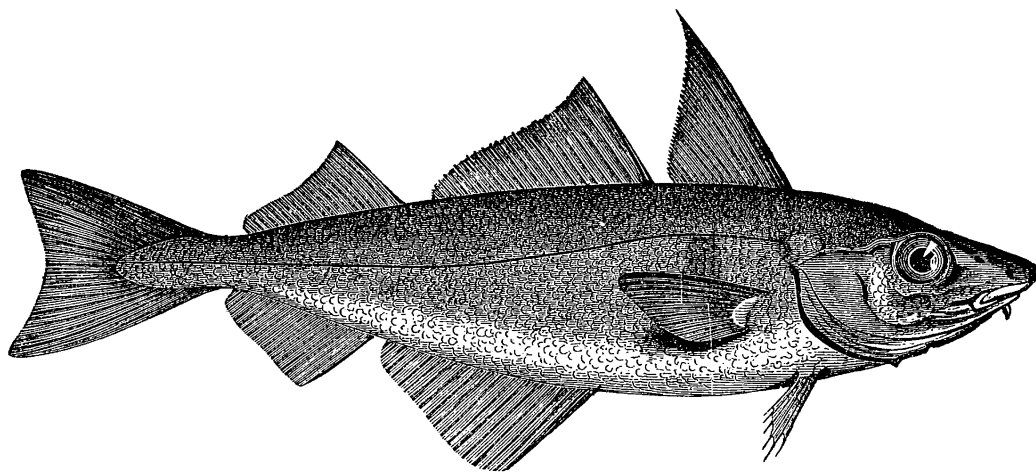
"If, during its stay in shallow water, the weather should suddenly become cold, and so remain for two or three days, the codfish immediately retreats to water of some forty fathoms in depth, and does not return till the temporary change has passed; then they gradually seek their former resort, which is a depth of fifteen or twenty fathoms. The Haddock [pictured] at such times likewise retreats, but does not so soon return to its former station." From "The Habits and Migrations of Some of the Marine Fishes of Massachusetts" by James H. Blake (*The American Naturalist*, 1870, 4:513–521).