
Temporally Variable Multivariate Sexual Selection on Sexually Dimorphic Traits in a Wild Insect Population.
Author(s): David Punzalan, F. Helen Rodd, and Locke Rowe
Reviewed work(s):
Source: *The American Naturalist*, Vol. 175, No. 4 (April 2010), pp. 401-414
Published by: The University of Chicago Press for The American Society of Naturalists
Stable URL: http://www.jstor.org/stable/10.1086/650719
Accessed: 01/10/2012 10:55

---

vol. 175, no. 4 the american naturalist april 2010 Ⓔ

Temporally Variable Multivariate Sexual Selection on Sexually Dimorphic Traits in a Wild Insect Population

David Punzalan,[*] **F. Helen Rodd, and Locke Rowe**

Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario M5S 3B2, Canada

Submitted July 22, 2009; Accepted October 8, 2009; Electronically published February 25, 2010

Online enhancements: appendixes. *Dryad data:* http://hdl.handle.net/10255/dryad.909.

abstract: A widely held view is that the strength and form of natural selection varies in time and space in response to varying ecological forces; however, adequate quantitative evaluations of this are relatively scarce. In this study, we measured the strength and form of sexual selection acting on a suite of male morphological traits in a wild ambush bug (*Phymata americana*) population at 10 sampling dates over 2 years. We tested the prediction that the strength and direction of sexual selection would be associated with one or more important ecological variables. We found that patterns of multivariate selection varied considerably over time, and even within a season. Yet, for this population, a sexually dimorphic color pattern trait was consistently a target of directional selection. The strength of sexual selection on this trait was related to both sex ratio and density, which is consistent with the idea that ecological factors can play an important role in generating patterns of sexual selection. We also demonstrate that the median strength of linear selection obtained from replicated cross-sectional methods was qualitatively similar to the estimates obtained from longitudinal methods, providing multiple lines of evidence that the evolution of sexual color dimorphism in this species is attributable to sexual selection.

Keywords: sexual selection, sexual dimorphism, phenotypic selection, fluctuating selection, sex ratio.

Introduction

Understanding the strength and form of selection in nature is of fundamental importance in evolutionary biology (Kingsolver et al. 2001; Hereford et al. 2004). While our collective understanding of the typical strength and form of selection nature has been advanced by a considerable number of empirical studies in nature (Conner 2001; Hoekstra et al. 2001; Kingsolver et al. 2001), we still have only a limited understanding of how much and why patterns of selection vary (Arnold et al. 2001). It is typically assumed that the form and strength of selection on a given trait is determined by a suite of ecological variables. It is also generally assumed that these ecological factors may vary considerably over time and space, ultimately causing the form, direction, and strength of selection to vary accordingly (Wright 1932; Simpson 1953; Whitlock 1997). A number of phenotypic selection studies have demonstrated selection that varies in space and/or time (e.g., Kalisz 1986; Gibbs and Grant 1987; Weis et al. 1992; Milner et al. 1999; Preziosi and Fairbairn 2000; Kruuk et al. 2001; Grant and Grant 2002; Reimchen and Nosil 2002; Caruso et al. 2003; Coulson et al. 2003; Svensson and Sinervo 2004; Chaine and Lyon 2008; Gosden and Svensson 2008; Kasumovic et al. 2008). However, the majority of these considered only selection on a single trait or ignored selection on trait variances and covariances. Although these studies are valuable contributions to understanding variability in patterns of natural selection, selection is not restricted to linear selection acting on single traits in isolation, as it is more likely to act on multiple traits simultaneously and, possibly, on trait combinations (e.g., Lande and Arnold 1983; Phillips and Arnold 1989; Brodie 1992; Schluter and Nychka 1994; Blows and Brooks 2003; Blows 2007).

Lande and Arnold (1983) introduced a powerful framework for estimating and understanding multivariate selection. Estimable coefficients of linear and nonlinear selection describe selection acting on trait means, variances, and covariances, and they effectively approximated the selective landscape (sensu Wright 1932; Simpson 1953; see also Schluter 1988). Features of the selective landscape may be temporally dynamic; the degree and scale of temporal variability (both within and between generations) has crucial implications for a number of fundamental issues in evolutionary quantitative genetics, including the maintenance of genetic variation (Hedrick 1986; Gillespie and Turelli 1989; Ellner and Hairston 1994; Turelli and Barton 2004), genetic architecture (Bulmer 1980; Turelli 1988; Kawecki 2000; Jones et al. 2004), and, ultimately, the rate and direction of phenotypic evolution (Hansen and Houle

---

[*] Corresponding author. Present address: Centre for Ecology and Conservation, University of Exeter in Cornwall, Penryn, Cornwall TR10 9EZ, United Kingdom; e-mail: dpunzalan@mac.com.

Am. Nat. 2010. Vol. 175, pp. 401–414.
DOI: 10.1086/650719

2004; Estes and Arnold 2007; Lande 2007). However, the degree to which the features of the selective surface (and the adaptive landscape) vary (or are conserved) over ecological and evolutionary timescales remains a largely unexplored empirical question (Arnold et al. 2001; but see Rundle et al. 2008).

Temporal changes in patterns of selection may reflect variability in the position of a selective optimum. If such changes are principally dictated by ecological variables that determine the variance in relative fitness among individuals and/or the selective value of traits, then the strength of selection may vary predictably according to ecological factors (e.g., Gibbs and Grant 1987; Reimchen and Nosil 2002; Caruso et al. 2003; Twiss et al. 2007). Excellent examples of how such factors affect variance in fitness and, subsequently, the strength of selection can be seen in the dynamics of mating systems, where both abiotic and biotic factors can exert considerable influence. Climatic variables have been linked to variation in both the opportunity for sexual selection and the strength of sexual selection (Blanckenhorn et al. 1999*b*; Jann et al. 2000). Similarly, demographic factors such as operational sex ratio and population density have been shown to affect the strength of sexual selection because of their relation to the spatial distribution and density of prospective mates and, consequently, the intensity of competition for mates (Emlen and Oring 1977; Sutherland 1985; Hubbell and Johnson 1987; Conner 1989; Arnqvist 1992; McLain 1992; Carroll and Salamon 1995; Coltman et al. 1999). Finally, variability in selection can complicate the interpretation of selection coefficients, depending on the method of measurement. Phenotypic selection coefficients can be estimated from populations where relative fitness is measured from either longitudinal or cross-sectional data (Lande and Arnold 1983; Arnold and Wade 1984*b*). Both methods appear to yield estimates of similar magnitude (Kingsolver et al. 2001); however, the latter method is potentially misleading if the interval during which selection is estimated fails to represent selection acting throughout the lifetime of individuals in the population (Arnold and Wade 1984*a*). An intuitive expectation would be that, for a given population, the central tendency of coefficients derived from multiple cross-sectional estimates would approach the values obtained from a longitudinal estimate. However, the degree to which temporally replicated cross-sectional estimates of selection are in agreement with longitudinal estimates of selection for a given population has not, to our knowledge, been investigated beyond partitioning of selection according to different episodes or components of fitness (e.g., Arnold and Wade 1984*a*; Moore 1990; Schluter et al. 1991; Nishida 1994). On the other hand, however, cross-sectional data can be very informative for understanding the degree to which selection pressures vary over time as well as for identifying the causal ecological agents that underlie this variability; one potential drawback of relying on only longitudinal analyses is that it describes the average relationship between character(s) and fitness and does not provide information on the degree of variability in this relationship during the lifetime of an individual (i.e., within-generation variability).

In this study we use both longitudinal and cross-sectional analyses as complementary approaches to evaluate patterns of sexual selection in a wild population of ambush bugs (*Phymata americana*). Previous work on the same population of *P. americana* has revealed evidence of sexual selection on components of male color pattern (Punzalan et al. 2008*c*). Both the form and the strength of selection varied between sampling dates within a season, but the study was limited to two sampling dates and could not assess whether this variation was related to ecological differences. A subsequent study of the mechanism of sexual selection in this species suggested that dark male lateral color pattern (described in "Methods") is a thermoregulatory adaptation that is favored because it enhances mate search (Punzalan et al. 2008*b*). Here we report variation in patterns of selection measured on 10 dates over two seasons (with one generation per season) to evaluate the degree to which the patterns of multivariate linear selection and nonlinear selection varied within and between seasons. We also evaluate the degree to which the strength of sexual selection on male color pattern was associated with several potentially important ecological variables, namely, sex ratio, density, and ambient temperature. Finally, we ask how phenotypic selection estimates from cross-sectional methods compare with those from longitudinal methods of estimation, and we relate these findings to the observed pattern of sexual dimorphism in this species.

Methods

Study Organism and Traits Measured

Selection was measured in males in a naturally occurring population of *Phymata americana* (Heteroptera: Phymatidae) at the Koffler Scientific Reserve at Joker's Hill, King, Ontario, Canada (44°03′N, 79°29′W). These insects are characterized by striking sexual dimorphism in color pattern and body size. Except where noted, we focused on four male traits: area of dark dorsal color pattern (dark dorsal color is apparent in both sexes but males are darker), area of lateral color pattern (apparently expressed only in males), pronotum width (a measure of linear size), and weight (both a correlate of three-dimensional size and, possibly, an index of condition). Previous work (Punzalan et al. 2008*b*, 2008*c*) has demonstrated significant sexual selection favoring dark lateral color pattern in males, possibly accounting for the observed sexual dimorphism in color pattern.

Cross-Sectional Sampling

Six cross-sectional samplings (on August 13, August 20, August 27, September 3, September 10, and September 17) were performed in 2003 at the same field site, and four samplings (on August 19, August 24, September 1, and September 10) were performed in 2004. All samples were taken on warm sunny days, the conditions under which mating activity occurs. Within each year, the selected sampling dates effectively spanned the length of the season when bugs were in the adult stage and potentially mating. The samplings were intentionally spaced approximately 1 week apart. The discrepancy in season length between years appeared to be the result of relatively later emergence of adults in 2004, possibly due to a cooler, wetter summer than in 2003 (D. Punzalan, unpublished data). At each sampling date, an independent measure of sex ratio and population density was obtained at around 11:00 (*P. americana* are relatively inactive until around midday, when ambient temperatures are quite warm; Balduf 1941) by taking a census of the numbers of single males, single females, and mating pairs (i.e., those copulating or in a precopulatory, coupled position; see Punzalan et al. 2008*c*) observed over a 30-min haphazard walk through the field site. Before copulation, males ride on the dorsal surface of females (i.e., coupling), apparently in a mate-guarding position. Because coupling persists for several hours and is highly correlated with copulation success (Punzalan 2007; Punzalan et al. 2008*c*), we used coupling frequency as a surrogate measure of mating success and as our measure of absolute fitness in our selection gradient analyses, discussed below. Within 4 h after the population census, single and mating males were collected; to obtain a balanced representation of both fitness classes, we attempted to collect comparable numbers of each. Minimum and maximum ambient temperatures were recorded with a digital thermometer during the sampling period. Insects were collected in individual plastic containers and transported live to the laboratory (University of Toronto), and they were stored overnight in an environmentally controlled room (27° ± 3°C under 14L : 10D). The following morning, bugs were weighed to the nearest 0.01 mg and photographed under standardized lighting conditions with a lightproof box illuminated by an LED light (Nikon SL-1) mounted on a digital camera (Nikon CoolPix 4500). Bugs were subsequently fed approximately four live adult fruit flies (*Drosophila melanogaster*), transported to the original field site, and released the next day. Digital images were later analyzed using Scion Image software (http://www.scioncorp.com) to measure pronotum width and the area of dark dorsal and dark lateral color patterns (hereafter, referred to as dorsal and lateral, respectively). Because *P. americana* is univoltine, replicate samples taken within each year can be considered as snapshots of selection acting on a given population of individuals at different times within the same generation. Phenotypic selection coefficients were calculated for each sampling date; mating success (defined as a male successfully copulating or coupled with a female) was used as the measure of absolute fitness. The full details regarding the calculation of phenotypic selection gradients are described below.

Longitudinal Sampling

In 2003 we conducted a mark-release-recapture study on a field site located approximately 500 m from the site we used for cross-sectional studies. The chosen site was a rectangular field (90 × 30 m), with vegetation similar to that of the site used for cross-sectional studies, and it was partially surrounded on three sides by forest or rows of trees and tall bushes. In the summer of 2002, an array of 15 parallel transects spaced 5 m apart (marked with 1.5-m-tall wooden stakes that were spaced 5 m apart along each transect) was established; this array spanned most of the field. We conducted the mark-release-recapture study by marking and photographing bugs as they were encountered along each transect, as well as noting whether the observed males were single or mating (coupled or copulating). The order of transects sampled was randomized, and all photography was conducted in situ using a camera and lighting setup identical to those used in the cross-sectional analyses. Individuals were marked with tags bearing a unique three-digit identification number that was printed in black, 2-point Times New Roman font on white copy paper and protected by a layer of adhesive transparent laminate. Tags were precut to a size of 1 × 1.5 mm and stored in a centrifuge tube until their application. A custom-built device was employed to gently restrain bugs until tags could be mounted with Krazy Glue Gel to the dorsal surface of the pronotum using fine forceps. Although we were somewhat concerned that the placement of the tags could interfere with the natural dorsal color pattern phenotype and mobility, pilot studies indicated that it was the most suitable location. Its location also prevented it from being rubbed off by the hind legs while making it easily visible without having to handle the bugs during sampling. Males were photographed on first capture and, because the color pattern of *P. americana* darkens with age, upon capture at 14 days of age or older (estimated from date of first sighting and date of last sighting). A previous laboratory study revealed that darkening appears to asymptote between the ages of 10 and 17 days (Punzalan et al. 2008*a*). We did not measure male weight due to the practical difficulty associated with obtaining precise measurements in the field. The longitudinal field study was conducted on 28 days (roughly every other day) during a period between July 24 and September 26, 2003, corresponding to the approximate dates of earliest and final sighting of adults at the site. We performed daily walks along transects for several days after September 26 but did not encounter any bugs, which suggests that population densities were extremely low and, likely, that the reproductive season had ended at that site. Although the distance between cross-sectional and longitudinal sites probably did not exclude migration between sites, in only two instances over two seasons did we observe marked males from the longitudinal sampling at the site used for cross-sectional sampling.

At the end of the season, we analyzed digital images to measure pronotum width and dorsal and lateral color patterns in a manner identical to that described for the cross-sectional sampling study. For measures of dorsal color pattern, we excluded the area occupied by the identification tag. We also compiled a summary score of mating success for each marked male, defined as the proportion of sightings in which a male was observed to have been mating (i.e., coupled or copulating). The distribution of number of sightings (i.e., recapture rates) for each male was centered at a median of 2 (maximum $= 9$; mean $= 2.64$). Because recapture rates probably reflected both mortality and short-distance dispersal, in this study we did not estimate selection on the basis of survival; fitness was estimated on the basis of mating success. To minimize the influence of individuals sighted very few times (individuals who were sighted only once had a score of 0.00 or 1.00), we restricted our analyses to individuals sighted a minimum of three times. Thus, a total of 233 males throughout the 2003 season were marked, but only those that were sighted at least three times and had color pattern measurements at a minimum age of 14 days were included in the longitudinal phenotypic selection analysis ($n = 44$ males). The mean (SD) longevity of all males ($n = 233$) on the site (i.e., calculated from first and last date of recapture) was 10.62 (11.14), and the mean proportional mating success for males sighted a minimum of three times ($n = 53$) was 0.377 (0.379).

Estimation of Selection Coefficients

For each cross-sectional sample (2003 and 2004), we calculated standardized linear selection gradients (β) and nonlinear selection gradients (γ) for the four measured male traits using multiple linear regression (Lande and Arnold 1983). Within each sample, each trait was standardized to a zero mean and unit variance and the fitness of each individual was scored according to relative fitness (individual absolute fitness divided by mean fitness; Lande and Arnold 1983; Brodie et al. 1995). Univariate nonlinear selection gradients were obtained by doubling the quadratic coefficients of the second-order regression model, as outlined in Stinchcombe et al. (2008). Because the measure of fitness was binary (mated vs. unmated), gradients were estimated using linear regression but significance testing for the selection gradients was performed using logistic regression (Janzen and Stern 1998). We assessed significance of gradients at the conventionally accepted value $\alpha = 0.05$ as well as at $\alpha = 0.005$ to adjust for the multiple samples (i.e., 10 separate estimates). In addition, because classes of individuals may not have been sampled according to their actual abundance (Arnold and Wade 1984*b*), we applied the appropriate correction to the estimated coefficients according to the equations outlined by Blanckenhorn et al. (1999*a*).

For the longitudinal study (2003), we calculated standardized linear selection gradients (β) and nonlinear selection gradients (γ) for the same suite of male traits except for weight (which was not measured). Absolute fitness (proportion of recaptures where a male was mating) was first arcsine–square root transformed before calculating relative fitness. Coefficients and their significance were estimated using multiple linear regression. The opportunity for selection (I) was calculated as the variance in relative fitness (Arnold and Wade 1984*b*).

Assessing Temporal Variability in Patterns of Multivariate Selection

To evaluate the variability of patterns of linear selection within and between cross-sectional sampling periods (i.e., weeks and years), we used a mixed-model multiple logistic regression (in JMP), where mating success was a binary response variable and the four measured male traits were independent (continuous) variables. This analysis was performed for the data pooled across sampling dates but with trait standardizations calculated for each sample separately. Year, week (nested in year), and the trait × year and trait × week(year) interactions were also included as categorical (dummy) variables. Following the convention of measuring linear and nonlinear selection gradients in separate models (Lande and Arnold 1983), we constructed a similar model (with cross-product trait terms and their interactions with year and week (nested in year) to assess variability in patterns of nonlinear selection.

To aid in interpretation, we visualized temporal variability in patterns of selection using a geometric approach. To evaluate variability of features of the individual surface within and between years, we treated the data for linear and nonlinear selection separately. In addition to the coefficients estimated from the 10 cross-sectional samples obtained in this study, for the visualizations we included estimates of linear and nonlinear selection from two additional cross-sectional estimates obtained from the same population as that in a previous study in 2002 (Punzalan et al. 2008*c*).

To compare the vectors of linear selection on male traits among the 12 cross-sectional samples, we measured the closest angle (φ) between each pair of column vectors (normalized to unit length) in trait space according to

$$\varphi = \cos^{-1} |\beta_j^T \beta_k|,$$

where j and k indicate different sampling dates and the superscript T indicates the matrix transpose. Note that this approach constrained angles to vary between 0° (indicating that axes of linear selection are identical) and 90° (indicating that axes of linear selection are orthogonal). A 12×12 dissimilarity matrix **B** was constructed such that the elements are the angles corresponding to each pair of samples that were compared. We performed a principal coordinates analysis on matrix **B** and plotted the 12 observations (samples) onto the same multivariate space defined by the first two principal coordinate axes. The concordance of β vectors was assessed qualitatively by visual inspection for clustering of vectors according to sampling year and interpreted as indicative of variability in linear selection within and between years.

We used an analogous approach to visualize temporal variability in nonlinear selection (i.e., the gamma matrix). We used a series of multivariate analyses to visually assess and simultaneously compare the 12 estimated gamma matrices. We first employed a canonical rotation of each gamma matrix to reduce the information to four principal axes (eigenvectors $\mathbf{m}_1$–$\mathbf{m}_4$) in each sample. Canonical rotation of the γ-matrix effectively identifies the major axes of nonlinear selection (i.e., curvature of the selective surface), and it has a number of computational advantages because of its ability to reduce the number of dimensions and to reveal selection acting on trait combinations beyond merely pairwise comparisons (Phillips and Arnold 1989; Blows and Brooks 2003; Blows 2007).

Scree plots of the distributions of eigenvalues for each rotated gamma matrix revealed considerable variability among samples, often with similar amounts of variance summarized along several eigenvectors. We retained the first three eigenvectors (i.e., with the largest absolute eigenvalues) for each sample in subsequent analyses. For each pair of samples, we assessed pairwise concordance of fitness surfaces (which we defined by the orientation of the first three eigenvectors) using PROTEST, a procedure that uses Procrustean superimposition to calculate geometric concordance of a pair of matrices (Gower 1971; Jackson 1995; Peres-Neto and Jackson 2001). This procedure translates, stretches, and rotates one matrix with reference to a second reference matrix to compare, on the basis of least squares residuals among points (or vectors), the similarity of configurations in terms of the dimensions (e.g., trait axes) considered.

PROTEST calculates the statistic $\mathbf{m}_{12}$, which can be considered to be a measure of matrix dissimilarity based on orthogonal least squares residuals. We used the $\mathbf{m}_{12}$ values to construct a dissimilarity matrix **D**, which was subsequently subjected to a principal coordinates analysis. The concordance of (the first three eigenvectors of) the gamma matrices was visualized by projecting the samples onto the same space, defined by the first two principal coordinate axes of the distance matrix **D**.

Comparing Cross-Sectional and Longitudinal Estimates of Selection

To evaluate whether multiple cross-sectional estimates of selection can approximate those obtained from longitudinal estimates, we compared the selection gradients from the cross-sectional samples obtained in 2003 with the selection gradients from the longitudinal study in the same year. Since selection gradients are estimated from multiple regressions, the estimated partial regression coefficients are somewhat dependent on which traits/variables are included in the model (Lande and Arnold 1983; Mitchell-Olds and Shaw 1987). Because a different number of traits were included in the cross-sectional (four traits) and the longitudinal (three traits) studies, for the comparison of the two methods we used recalculated cross-sectional selection gradients, with weight excluded from the regression models. For each trait separately, we calculated the median standardized selection linear (β_{med}) and nonlinear (γ_{med}) gradients for the six cross-sectional samples in 2003 and compared them with the estimates of β and γ measured from the longitudinal study in the same year. The comparisons between longitudinal and cross-sectional estimates were of a qualitative nature, because the estimates of selection are dependent on the chosen scale for scoring fitness (Brodie and Janzen 1996). Since the measure of fitness in the cross-sectional studies was binary (mated vs. unmated) while the measure of fitness in the longitudinal study was based on proportional mating success, we were specifically interested in whether longitudinal and cross-sectional methods produced coefficients of similar direction rather than of magnitude.

Ecological Predictors of the Strength of Sexual Selection

We tested whether the strength and direction of linear sexual selection on lateral color pattern (previously established as a sexually selected trait; Punzalan et al. 2008*b*,

Table 1: Standardized selection gradients for male *Phymata americana* and demographic parameters from cross-sectional samples taken in 2003

			γ					
Sample, trait	Mean (SD)	β	PN	WT	DO	LA	Sex ratio	Density
Sample 1 ($N = 94$)							.68	129
PN	3.02 (.15)	.138	.213					
WT	19.6 (3.8)	−.325[a]	−.239	.539[a]				
DO	15.85 (2.97)	.418	−.300	.328	.030			
LA	4.37 (2.30)	.233	.318	−.223	.243	.382		
Sample 2 ($N = 115$)							.98	115
PN	3.01 (.16)	.056	.049					
WT	22.4 (4.8)	−.073	−.042	.236				
DO	15.83 (2.86)	−.244[b]	−.119	−.228	.185			
LA	4.13 (1.20)	.258[b]	.335	.095	−.321	.342		
Sample 3 ($N = 102$)							1.13	179
PN	3.03 (.16)	.107	.278					
WT	19.7 (3.02)	−.041	−.152	−.097				
DO	16.22 (2.03)	−.285[b]	.052	.037	−.070			
LA	5.02 (1.37)	.271[b]	−.314	.529[a]	.185	−.025		
Sample 4 ($N = 121$)							1.16	147
PN	3.02 (.16)	−.032	.082					
WT	13.9 (2.9)	−.042	−.117	.070				
DO	16.80 (2.09)	−.088	−.144	.107	−.138			
LA	5.09 (1.00)	.118	.097	−.117	−.075	.176		
Sample 5 ($N = 102$)							1.14	79
PN	3.04 (.18)	−.291	−.450					
WT	18.7 (3.6)	.193	.028	−.095				
DO	16.59 (2.03)	.067	.199	.003	.527[a]			
LA	5.59 (1.10)	−.008	.068	.096	.318	−.228		
Sample 6 ($N = 67$)							2.14	22
PN	3.09 (.17)	−.159	.307					
WT	16.9 (3.2)	.163	−.009	.175				
DO	17.50 (1.72)	.554	−1.087[a]	−.109	1.084			
LA	6.03 (.70)	−.465	.592[a]	.163	.223	−.681[a]		

Note: Standardized linear (β) and nonlinear (γ) selection gradients are measured for pronotum width (PN), weight (WT), and dorsal (DO) and lateral (LA) color patterns. Also reported are the trait means and SDs before standardization, sex ratios, and relative abundances. Trait means are reported in millimeters (for PN), milligrams (for WT), and square millimeters (for DO and LA). Sex ratio (males : females) and density (total number of adults) measurements are based on a 30-min census performed at the site before each sampling event. Gradients were estimated using linear multiple regression; significance testing was performed using logistic regression. Corresponding standard errors are reported in table A1 in the online edition of the *American Naturalist*.

[a] Significance at $\alpha = 0.05$.

[b] Significance at $\alpha = 0.005$.

2008*c*) covaried with the ecological factors likely to influence mating patterns: population density and sex ratio. Because of limited statistical power, we did this in a simple bivariate manner (i.e., rather than in a multiple regression), separately plotting the relationship between the linear selection gradients estimated for lateral color pattern (β_{lateral}) at each cross-sectional sample and either demographic variable. For the analysis of sex ratio, we included the 2003 and 2004 samples plus two additional cross-sectional estimates from a previous field study performed at the same site in 2002 ($n = 12$; Punzalan et al. 2008*c*). Note that the estimates of sex ratio in 2002 were based on the actual representation of males and females in the samples (bugs were sampled according to their abundance), unlike in the 2003–2004 samples, where sex ratio was estimated from a census that was performed before sampling. For the analysis of population density, we did not include the data from 2002 because of differences in sampling methods that make it difficult to directly compare the estimates of density in 2002 with those in 2003–2004 ($n = 10$). Because the sexually selected trait in *P. americana* has been linked to thermoregulation (Punzalan et al. 2008*b*), we examined the bivariate relationship between the strength of linear selection on lateral coloration

Table 2: Standardized selection gradients for male *Phymata americana* and demographic parameters from cross-sectional samples taken in 2004

			γ					
Sample, trait	Mean (SD)	β	PN	WT	DO	LA	Sex ratio	Density
Sample 7 (*N* = 146)							1.55	148
PN	3.07 (.15)	−.024	−.112					
WT	17.6 (3.0)	.305[a]	.299	.202				
DO	17.12 (2.28)	.764[b]	.005	.128	.329			
LA	4.97 (1.65)	−.468[a]	.472	.067	−.171	−.283		
Sample 8 (*N* = 189)							1.39	134
PN	3.05 (.14)	.122	.181					
WT	18.0 (4.8)	.069	−.049	.243				
DO	12.77 (3.13)	.017	−.008	.119	−.116			
LA	3.44 (1.55)	.565[b]	.179	.083	.112	.167		
Sample 9 (*N* = 105)							1.31	148
PN	3.07 (.15)	.390[b]	.254					
WT	21.4 (2.0)	−.104	.039	−.157				
DO	15.64 (2.04)	−.520[b]	−.329	.408	.355			
LA	5.35 (.69)	.350[b]	.239	−.217	−.323[a]	.282[a]		
Sample 10 (*N* = 60)							1.75	66
PN	3.08 (.16)	−.123	−.146					
WT	18.3 (2.1)	.738[a]	.249	.667				
DO	13.6 (1.40)	.122	−.086	−.022	.195			
LA	3.89 (.72)	−.222	−.295[a]	.478[a]	−.022	−.545		

Note: Standardized linear (β) and nonlinear (γ) selection gradients are measured for pronotum width (PN), weight (WT), and dorsal (DO) and lateral (LA) color patterns. Also reported are the trait means and SDs before standardization, sex ratios, and relative abundances. Trait means are reported in millimeters (for PN), milligrams (for WT), and square millimeters (for DO and LA). Sex ratio (males : females) and density (total number of adults) measurements are based on a 30-min census performed at the site before each sampling event. Gradients were estimated using linear multiple regression; significance testing was performed using logistic regression. Corresponding standard errors are reported in table A2 in the online edition of the *American Naturalist*.

[a] Significance at $\alpha = 0.05$.

[b] Significance at $\alpha = 0.005$.

and two separate measures of ambient environmental temperature: minimum and maximum ambient temperatures recorded at the sampling site during collection.

Regression coefficients, gradients, and their significance were estimated using mixed-model linear and logistic regressions with JMP, version 4.0.3. Canonical rotation of the gamma matrices were performed using Poptools 2.6.2 (available at http://www.csiro.au/poptools). A Procrustes superimposition and permutation test was performed using PROTEST (Peres-Neto and Jackson 2001; available at http://labs.eeb.utoronto.ca/jackson/pro1.html), and principal coordinates analysis and visualization of dissimilarity matrices were performed using NTSYSpc, version 2.0.

Results

Temporal Variability in Patterns of Selection

The sign, strength, and form of sexual selection on each male trait varied considerably among the 10 cross-sectional samples (summarized in tables 1 and 2; see also tables A1 and A2 in the online edition of the *American Naturalist*). Among cross-sectional samples in both years, we detected 13 linear selection gradients that were significant at $\alpha = 0.05$, of which eight indicated positive linear selection and five indicated negative linear selection (at $\alpha = 0.005$, six were positive and three were negative). Notably, male color pattern trait was a prominent target of selection in a number of samples. We detected significant directional selection (at $\alpha = 0.005$) acting to favor increased expression of dark male lateral color pattern in four of the samples, although linear selection on this trait varied across samples (i.e., significant trait × sample interactions; table B1 in the online edition of the *American Naturalist*). Conversely, dorsal coloration was often subjected to negative linear selection (i.e., in three samples at $\alpha = 0.005$). Ten nonlinear selection gradients were significant at $\alpha = 0.05$, of which six were positive and four were negative. We did not detect significant nonlinear selection at $\alpha = 0.005$. The full model, however, did indicate significant temporal variation in patterns of nonlinear selection across samples within years (table B2 in the online edition of the *American Naturalist*). In examining the patterns of selection graphically, we found considerable variation in the

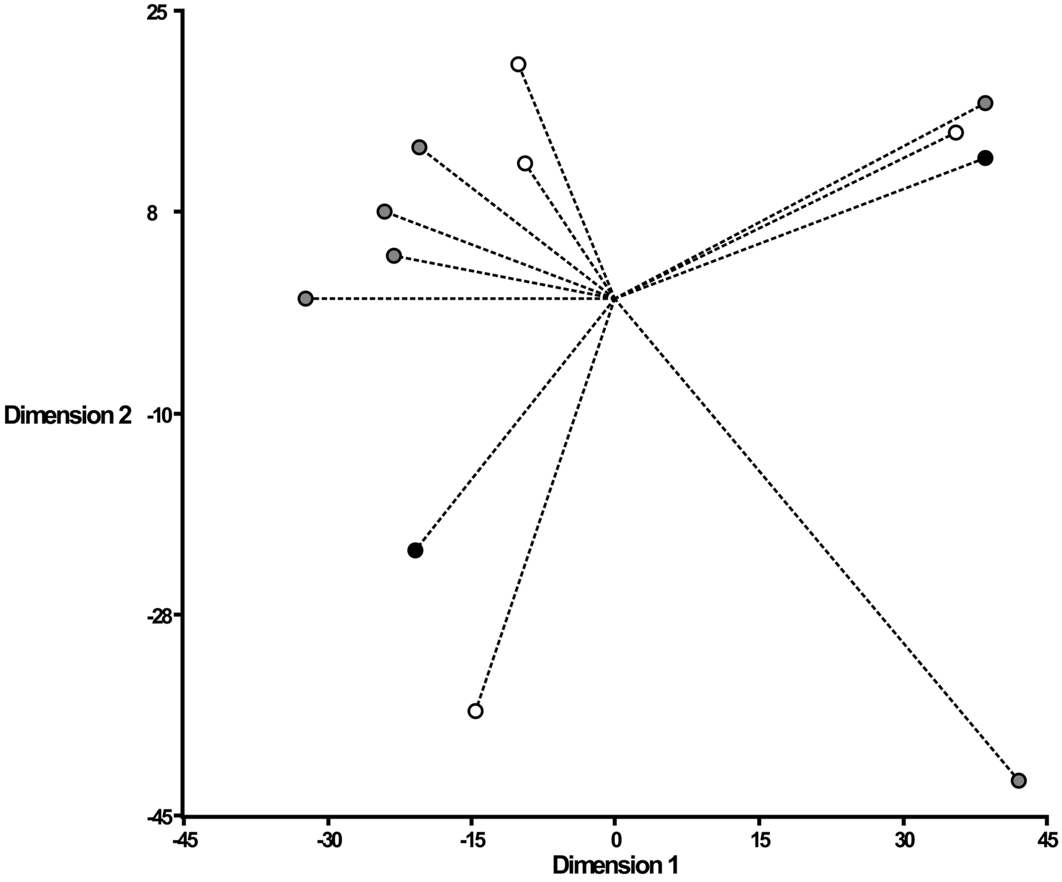

Figure 1: Projection of samples in two-dimensional principal coordinates space revealed no clustering of vectors of linear selection according to year. Multivariate distances were calculated on the basis of angles between vectors of linear selection (β) for each sample in 2002 (*black circles*), 2003 (*gray circles*), and 2004 (*open circles*).

angle between pairs of vectors of linear selection among samples, suggesting a large degree of temporal variability in patterns of linear selection. The lack of clustering of vectors indicated within-year variability in linear selection that was comparable to that observed between years (fig. 1). Similarly, patterns of nonlinear selection were highly variable both within and among years (fig. 2).

Comparison of Cross-Sectional and Longitudinal Estimates of Linear Selection

The linear selection gradients estimated from the longitudinal study were not statistically different from zero but exhibited a trend suggestive of positive linear selection on lateral color pattern ($\beta = 0.182$, $P = .088$; tables 3, 4). The median vector of linear selection estimated from cross-sectional estimates in 2003 (table 5) provided a qualitatively similar finding to the estimates derived from longitudinal methods in the same year. Both the median cross-sectional estimates and the longitudinal estimates suggest male lateral coloration as a target of positive directional sexual selection. For the nonlinear selection analyses, the concordance between the median estimates (table 5) from the cross-sectional analyses and the longitudinal data set was reasonably good, although there was one discrepancy. The longitudinal data set detected significant nonlinear selection on several traits/trait combinations, including convex (e.g., stabilizing) selection on dorsal color, but the median cross-sectional estimates indicated nonlinear selection of the opposite sign. The longitudinal analysis also suggested significant correlational selection favoring a negative covariance between dorsal color and pronotum width, a pattern that was reflected in the median estimates obtained from cross-sectional analyses.

Ecological Predictors of the Strength of Sexual Selection

The sign and strength of direct linear selection on lateral color pattern (β_{lateral}) showed a strong inverse relationship

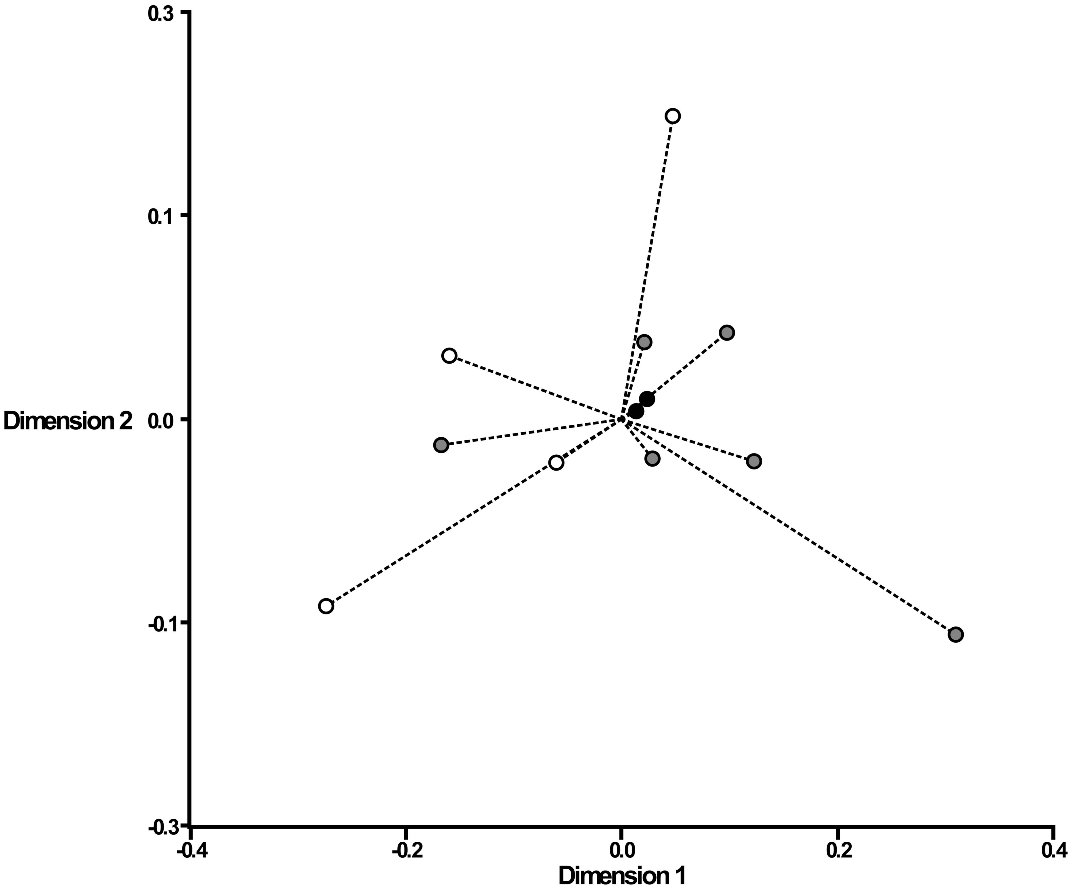

Figure 2: Projection of samples in two-dimensional principal coordinates space revealed no clustering on the axes of nonlinear selection according to year. Multivariate distances were calculated on the basis of $\mathbf{m}_{12}$ values from Procrustean superimposition for each pairwise comparison of the three principal axes of nonlinear selection (γ) for each sample in 2002 (*black circles*), 2003 (*gray circles*), and 2004 (*open circles*).

with sex ratio ($r = -0.658$, $P = .039$, $n = 10$; fig. 3*A*) across samples in 2003 and 2004; lateral color pattern was favored for female-biased sex ratios but selectively disfavored when sex ratio was male biased. This relationship was true even when two additional estimates of β_{lateral} from 2002 were included ($r = -0.696$, $P = .012$, $n = 12$). The opposite trend was observed in terms of population density, where there was a positive but not statistically significant association with β_{lateral} ($r = 0.569$, $P = .086$, $n = 10$; fig. 3*B*). There was no significant relationship between β_{lateral} and any measure of environmental temperature (minimum temperature: $r = 0.133$, $P = .715$, $n = 10$; maximum temperature: $r = 0.280$, $P = .434$, $n = 10$). Similar bivariate comparisons with dorsal color pattern showed no significant trends.

Discussion

Patterns of selection are expected to vary over time as a result of varying ecological conditions; this can have a number of important evolutionary consequences. This variation also poses a challenge to the accurate estimation of selection because a single estimate need not reflect the average strength or direction of selection. Few studies have adequately characterized temporal variation in selection, and, similarly, evidence of a link between temporal variability and ecological factors is sparse. In this study we found that the strength of linear sexual selection on male-limited coloration varied significantly and that some of this variation could be attributed to population sex ratio and, to a lesser extent, population density. We also provide a rare comparison of estimates of selection obtained from

Table 3: Standardized selection gradients for male *Phymata americana* in a longitudinal phenotypic selection study in 2003

			γ		
Trait	Mean (SD)	β	PN	DO	LA
PN	3.01 (.171)	−.046	.032		
DO	13.64 (2.81)	−.059	−.267[a]	−.186[b]	
LA	4.69 (1.20)	.182[c]	.260[c]	.169	.226

Note: Standardized linear (β) and nonlinear (γ) selection gradients are measured for pronotum width (PN) and dorsal (DO) and lateral (LA) color patterns. Trait means are reported in millimeters (for PN) and square millimeters (for DO and LA). Also reported was the total opportunity for selection ($I = .642$) based on males sighted a minimum of three times ($n = 53$). Estimates are derived from $n = 44$ individually marked males (see "Methods"). Corresponding standard errors are reported in table 4.

[a] Significance at $\alpha = 0.005$.

[b] Significance at $\alpha = 0.05$.

[c] $.05 < P < .10$.

two alternative methods of estimating phenotypic selection. Both cross-sectional and longitudinal estimates of selection indicated overall positive directional selection on sexually dimorphic coloration. Our results are discussed in detail below, as they pertain to evolution of dimorphism in this species and to the broader context of quantifying and understanding evolutionary processes.

Overall Patterns of Sexual Selection

The strength, form, and even sign of sexual selection were highly variable among the cross-sectional samples. We primarily detected significant linear selection; nonlinear selection gradients were less frequently statistically significant, and only at the less conservative value $\alpha = 0.05$. It is difficult to discern whether this is indicative of a truly less common (or weaker) form of selection or merely an artifact of low statistical power associated with nonlinear estimates (Kingsolver et al. 2001). Among the 10 cross-sectional samples in 2003 and 2004, male lateral color pattern was often subjected to strong, positive directional (i.e., linear) selection. We also detected direct linear selection on lateral color in the longitudinal study, although the estimates were not quite significant, possibly because of low statistical power. Despite the variation among samples, this overall pattern of selection is consistent with previous manipulative laboratory studies demonstrating that dark lateral coloration is a target of sexual selection through its effects on mate searching (Punzalan et al. 2008*b*).

In contrast to our results for lateral color, our data suggest negative nonlinear selection (i.e., stabilizing; from the longitudinal analysis) or negative linear selection (i.e., from the cross-sectional analyses) on dorsal color. The dorsal color pattern is exaggerated in males compared with females, although less so than lateral color (Punzalan et al. 2008*a*). In light of the presence of negative or stabilizing selection on this trait in males, it is possible that the persistence of this sexual dimorphism in dorsal color pattern reflects some evolutionary constraint. One possibility is that dorsal color is genetically correlated with lateral color, thereby precluding advantageous combinations of high trait values for lateral color and low values for dorsal color pattern. There is some indirect evidence that suggests the potential for constraint; lateral color pattern and dorsal color are indeed phenotypically correlated (Punzalan et al. 2008*a*, 2008*c*), and the expression of both traits is similarly affected by resource limitation (i.e., both traits are condition dependent; Punzalan et al. 2008*a*), suggesting a shared physiological or developmental basis. Further study is required to determine whether genetic correlations constrain the independent evolution of these traits.

Temporal Variability in Patterns of Selection and Its Ecological Basis

Temporal variation in patterns of selection can have important implications for a number of central issues, including the direction and rate of evolution and the maintenance of genetic variation (e.g., Hedrick 1986; Ellner and Hairston 1994; Jones et al. 2004; Turelli and Barton 2004); however, our understanding of this variation is limited (Endler 1986; Kingsolver et al. 2001). In this study we evaluated temporal variation in patterns of both linear (β) and nonlinear (γ) selection in separate analyses, and both varied considerably. In short, sexual selection on male trait means and variances were highly variable over time. Selection fluctuated within year (generation), but the range of variation was similar among years. Although the consequences of fluctuating selection have often been considered in terms of between-generation variation in selection (e.g., Hedrick 1973; Ellner and Hairston 1994), less attention has been paid to the implications of within-generation variability. Both scales of temporal variation in selection, however, can have important consequences in terms of maintaining or eroding allelic and quantitative genetic variation, depending

Table 4: Standard errors associated with the selection gradients for the longitudinal study in 2003 (reported in table 3)

		SE (γ)		
Trait	SE (β)	PN	DO	LA
PN	.091	.164		
DO	.073	.095	.088	
LA	.104	.144	.100	.152

Note: Standardized linear (β) and nonlinear (γ) selection gradients are measured for pronotum width (PN) and dorsal (DO) and lateral (LA) color patterns.

Table 5: Median selection gradients for male *Phymata americana* averaged over six cross-sectional phenotypic selection studies in 2003

		γ_{med}		
Trait	β_{med}	PN	DO	LA
PN	.017	.058		
DO	.006	−.104	.019	
LA	.183	.242	.124	.153

Note: Values were calculated from standardized linear (β) and nonlinear (γ) selection gradients derived from analyses on pronotum width (PN) and dorsal (DO) and lateral (LA) color patterns (i.e., weight excluded from multiple regressions).

on a suite of genetic and selective factors (Hedrick 1986; Gillespie and Turelli 1989; Turelli and Barton 2004).

It is commonly asserted that varying ecological factors underlie such temporal variability in selection (discussed in Whitlock 1997); however, strong support for this view is relatively sparse (e.g., Blanckenhorn et al. 1999*b*; Grant and Grant 2002; Gosden and Svensson 2008). Here we demonstrated that much of the variation in linear sexual selection is accounted for with local sex ratio and, to some extent, population density. These results are in accord with a few studies that have shown that demographic factors play a key role in determining the dynamics of insect mating systems and, thus, the strength of sexual selection (e.g., Conner 1989; Arnqvist 1992; Carroll and Salamon 1995; Fairbairn and Preziosi 1996; Jann et al. 2000; see also Kokko and Rankin 2006). In *Phymata americana*, the mating system resembles a scramble and male lateral color pattern has no apparent role in male-female interactions (i.e., signaling) or the outcome of direct male-male competition (Punzalan et al. 2008*c*). Instead, a male's mating success appears to be limited by his ability to search for mates, and dark lateral coloration confers thermoregulatory advantages in searching (Punzalan et al. 2008*b*). Temporal fluctuations in sex ratio and density are likely to exert strong effects on the efficacy of mate searching, resulting in variability in the strength of sexual selection on male lateral coloration. A full understanding of the proximate basis for the observed associations between each factor and selection is complicated by a negative relationship between sex ratio and population density ($r = -0.614$, $P = .059$, $n = 10$). Inferring a causal role of these ecological variables in influencing patterns of selection requires further study, ideally with direct, experimental manipulation of these factors.

Inference from Multiple Estimates of Selection

Longitudinal studies of phenotypic selection are generally considered to be preferable to cross-sectional studies because the former are thought to provide a more complete estimate of lifetime fitness (Arnold and Wade 1984*a*, 1984*b*; Clutton-Brock 1988). However, in many biological systems, longitudinal studies are not tractable because of nuances in biology or the chosen fitness metric. In some cases, for example, in highly mobile organisms where individual marking and tracking is not possible or where fitness is assigned as alive versus dead, cross-sectional studies provide the only alternative (Brodie and Janzen 1996). In our study we found that for one population in one particular year (2003), repeated cross-sectional estimates of linear selection and, to a limited extent, nonlinear selection converged on the estimates using longitudinal methods. Nevertheless, single cross-sectional estimates did not necessarily correspond to the longitudinal estimate, suggesting that multiple cross-sectional estimates are required for the summaries of selection that can be obtained from longitudinal studies.

In fact, for some questions, a series of cross-sectional studies of selection may actually offer more insight than longitudinal studies. For example, in our study, we evaluated the role of ecological variables in mediating selection on a trait of particular interest. As a result of systematic sampling and subsequent comparison between estimates of selection and measured ecological variables, we were able to detect strong associations between selection and ecological context, an insight that could not have been gained from our longitudinal data alone. That being said, one must exercise caution if only a few cross-sectional estimates are obtained, as the large range of temporal variation we observed among sampling dates suggests that a given cross-sectional estimate can give a distorted picture of average selection pressures, whether due to underlying ecological variables or to stochastic sampling error. The latter may be particularly problematic when sample sizes are small; Kingsolver et al. (2001) noted that, on the basis of previously published studies, the variance in the strength of selection ($|\beta|$) was negatively associated with sample size, suggesting that small sample sizes are prone to overestimation of the strength of selection due to sampling error. In our study, however, the absolute strength ($|\beta|$) of selection on lateral color pattern (in the cross-sectional samples) was not significantly correlated with sample size. Rather, the strength of selection tended to be positively associated with sample size for cross-sectional estimates in 2003 and 2004 ($r = 0.439$, $P = 0.204$, $n = 10$). This relationship may be partly driven, however, by an inherent association between population density and sample size resulting from the methodology we used here (i.e., our sample sizes were limited by actual abundance).

Conclusions

Our study evaluated patterns of multivariate phenotypic selection in a wild population of *P. americana*. While we

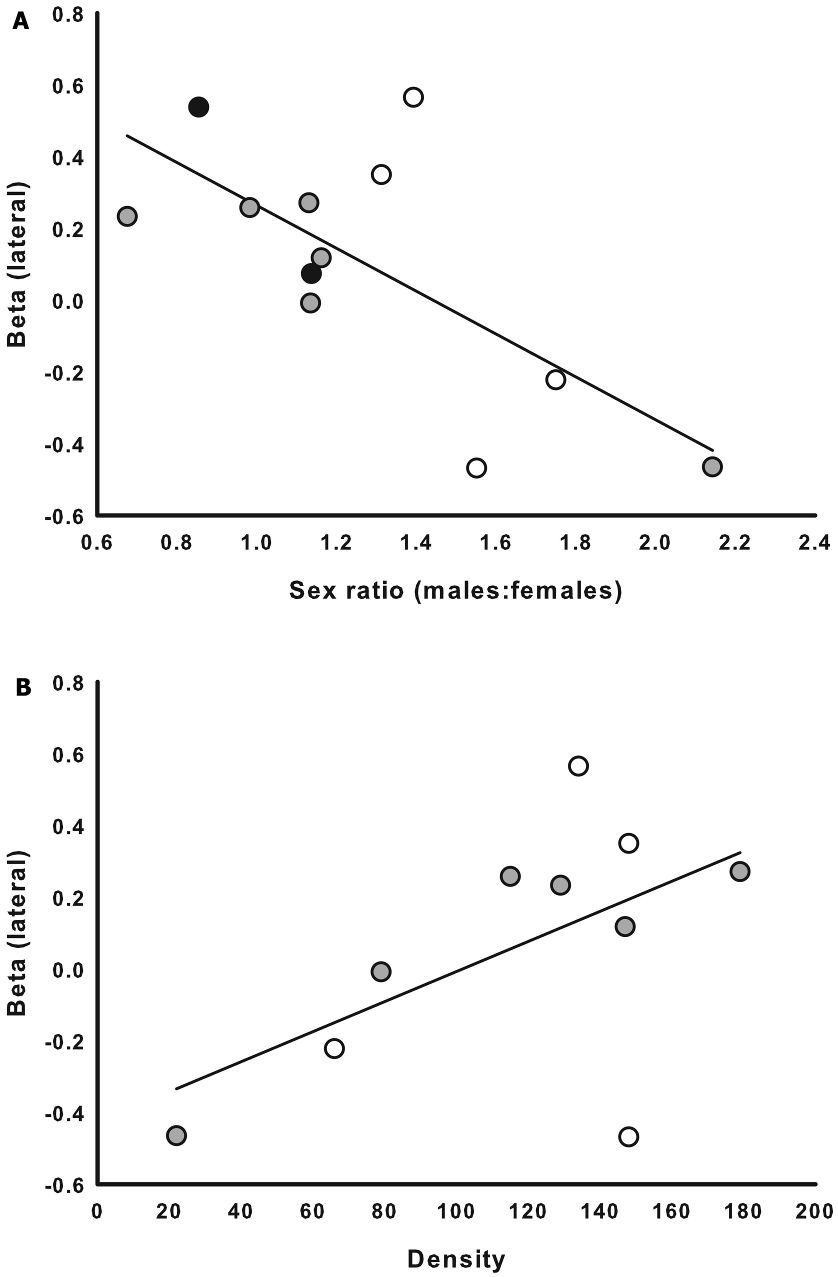

Figure 3: The strength of linear selection (β) corresponding to sex ratio (*A*) and population density (*B*) in samples collected in 2002 (*black circles*), 2003 (*gray circles*), and 2004 (*open circles*). Sex ratio and density were measured in a separate 30-min census conducted before sampling.

found that the features of the selective surface varied considerably both within and between generations, we frequently detected significant selection of the same sign on a sexually dimorphic male trait (lateral coloration). We also show that the sign and strength of sexual selection varied with important demographic variables, supporting the important role that ecology plays in determining mating system dynamics and the strength of selection. Furthermore, we compared estimates of phenotypic selection using two alternative methods (cross-sectional vs. longitudinal) and found that there was some qualitative concordance between the estimates; both suggest that sexual selection is responsible for the evolution and maintenance of the striking color pattern dimorphism in *P. americana.*

Acknowledgments

We thank M. Cooray for assistance in the field and laboratory. N. Mideo assisted with the analysis of color pattern data. We also thank M. Andrade, C. Caruso, J. Conner, D. Gwynne, J. Stinchcombe, and two anonymous reviewers for comments on previous versions of the manuscript. Thanks to D. Jackson and S. Sharma for statistical advice and to H. Rundle for stimulating discussion. This work was supported by funds from the Natural Sciences and Engineering Research Council (Canada) and the Premier's Research Excellence Awards (Ontario) to F.H.R. and L.R. D.P. was also supported by University of Toronto Fellowships and a W. John D. Eberlie Research Travel Award (Toronto Entomologists Association).

Literature Cited

Arnold, S. J., and M. J. Wade. 1984*a*. On the measurement of natural and sexual selection: applications. Evolution 38:720–734.

———. 1984*b*. On the measurement of natural and sexual selection: theory. Evolution 38:709–719.

Arnold, S. J., M. E. Pfrender, and A. G. Jones. 2001. The adaptive landscape as a conceptual bridge between micro- and macroevolution. Genetica 112/113:9–32.

Arnqvist, G. 1992. Spatial variation in selective regimes: sexual selection in the water strider, *Gerris odontogaster*. Evolution 46:914–929.

Balduf, W. V. 1941. Life history of *Phymata pennsylvanica americana* Melin (Phymatidae, Hemiptera). Annals of the Entomological Society of America 34:204–214.

Blanckenhorn, W. U., M. Reuter, P. I. Ward, and A. D. Barbour. 1999*a*. Correction for sampling bias in quantitative measures of selection when fitness is discrete. Evolution 53:286–291.

Blanckenhorn, W. U., C. Morf, C. Muhlhauser, and T. Reusch. 1999*b*. Spatiotemporal variation in selection on body size in the dung fly *Sepsis cynipsea*. Journal of Evolutionary Biology 12:563–576.

Blows, M. W. 2007. A tale of two matrices: multivariate approaches in evolutionary biology. Journal of Evolutionary Biology 20:1–8.

Blows, M. W., and R. Brooks. 2003. Measuring nonlinear selection. American Naturalist 162:815–820.

Brodie, E. B., III. 1992. Correlational selection for color pattern and antipredator behavior in the garter snake *Thamnophis ordinoides*. Evolution 46:1284–1298.

Brodie, E. B., III, and F. J. Janzen. 1996. On the assignment of fitness values in statistical analyses of selection. Evolution 50:437–442.

Brodie, E. B., III, A. J. Moore, and F. J. Janzen. 1995. Visualizing and quantifying natural selection. Trends in Ecology & Evolution 10:313–318.

Bulmer, M. G. 1980. The mathematical theory of quantitative genetics. Clarendon, Oxford.

Carroll, S. P., and M. H. Salamon. 1995. Variation in sexual selection on male body size within and between populations of the soapberry bug. Animal Behaviour 50:1463–1474.

Caruso, C. M., S. B. Peterson, and C. E. Ridley. 2003. Natural selection on floral traits of *Lobelia* (Lobeliaceae): spatial and temporal variation. American Journal of Botany 90:1333–1340.

Chaine, A. S., and B. E. Lyon. 2008. Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. Science 25:459–462.

Clutton-Brock, T. H. 1988. Introduction. Pages 1–6 *in* T. H. Clutton-Brock, ed. Reproductive success. University of Chicago Press, Chicago.

Coltman, D. W., J. A. Smith, D. R. Bancroft, J. Pilkington, A. D. C. MacColl, T. H. Clutton-Brock, and J. M. Pemberton. 1999. Density-dependent variation in lifetime breeding success and natural and sexual selection in Soay rams. American Naturalist 154:730–746.

Conner, J. 1989. Density-dependent sexual selection in the fungus beetle, *Boliotherus cornutus*. Evolution 43:1378–1386.

Conner, J. K. 2001. How strong is natural selection? Trends in Ecology & Evolution 16:215–217.

Coulson T., L. E. B. Kruuk, G. Tavecchia, J. M. Pemberton, and T. H. Clutton-Brock. 2003. Estimating selection on neonatal traits in red deer using elasticity path analysis. Evolution 57:2879–2892.

Ellner, S., and N. G. Hairston. 1994. Role of overlapping generations in maintaining genetic variation in a fluctuating environment. American Naturalist 143:403–417.

Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. Science 197:215–222.

Endler, J. A. 1986. Natural selection in the wild. Princeton University Press, Princeton, NJ.

Estes, S., and S. J. Arnold. 2007. Resolving the paradox of stasis: models with stabilizing selection explain evolutionary divergence on all timescales. American Naturalist 169:227–244.

Fairbairn, D. J., and R. F. Preziosi. 1996. Sexual selection and the evolution of sexual size dimorphism in the water strider, *Aquarius remigis*. Evolution 50:1549–1559.

Gillespie, J. H., and M. Turelli. 1989. Genotype-environment interactions and the maintenance of polygenic variation. Genetics 121: 129–138.

Gosden, T. P., and E. I. Svensson. 2008. Spatial and temporal dynamics in a geographic sexual selection mosaic. Evolution 62:845–856.

Gower, J. C. 1971. Statistical methods of comparing different multivariate analyses of the same data. Pages 138–149 *in* F. R. Hodson, D. G. Kendall, and P. Tautu, eds. Mathematics in the archaeological and historical sciences. Edinburgh University Press, Edinburgh.

Gibbs, H. L., and P. R. Grant. 1987. Oscillating selection in Darwin's finches. Nature 327:511–513.

Grant, P. R., and B. R. Grant. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. Science 296:707–711.

Hansen, T. F., and D. Houle. 2004. Evolvability, stabilizing selection and the problem of stasis. Pages 130–150 *in* M. Pigliucci and K. Preston, eds. Phenotypic integration. Oxford University Press, Oxford.

Hedrick, P. W. 1973. Genetic variation in a heterogeneous environment. I. Temporal heterogeneity and the absolute dominance model. Genetics 78:757–770.

———. 1986. Genetic polymorphisms in heterogeneous environments: a decade later. Annual Review of Ecology and Systematics 17:535–566.

Hereford, J., T. F. Hansen, and D. Houle. 2004. Comparing the strengths of directional selection: how strong is strong? Evolution 58:2133–2143.

Hoekstra, H. E., J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, P. Gilbert, P. Beerli, and J. G. Kingsolver. 2001. The strength and tempo of directional selection in the wild. Proceedings of the National Academy of Sciences of the USA 98:9157–9160.

Hubbell, S. P., and L. K. Johnson. 1987. Environmental variance in lifetime mating success, mate choice and sexual selection. American Naturalist 130:91–112.

Jackson, D. A. 1995. PROTEST: a Procrustean randomization test of community environment concordance. Ecoscience 2:297–303.

Jann, P., W. U. Blanckenhorn, and P. I. Ward. 2000. Temporal and microspatial variation in the intensities of natural and sexual selection in the yellow dung fly *Scathophaga stercoraria*. Journal of Evolutionary Biology 13:927–938.

Janzen, F. J., and H. S. Stern. 1998. Logistic regression for empirical studies of multivariate selection. Evolution 52:1564–1571.

Jones, A. G., S. J. Arnold, and R. Burger. 2004. Evolution and stability of the G-matrix on a landscape with a moving optimum. Evolution 58:1639–1654.

Kalisz, S. 1986. Variable selection on the timing of germination in *Collinsia verna* (Scrophulariaceae). Evolution 40:479–491.

Kasumovic, M. M., M. J. Bruce, M. C. B. Andrade, and M. E. Herberstein. 2008. Spatial and temporal demographic variation drives within-season fluctuations in sexual selection. Evolution 62:2316–2325.

Kawecki, T. J. 2000. The evolution of genetic canalization under fluctuating selection. Evolution 54:1–12.

Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, P. Gilbert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. American Naturalist 157:245–261.

Kokko, H., and D. J. Rankin. 2006. Lonely hearts or sex and the city? density-dependent effects in mating systems. Philosophical Transactions of the Royal Society B: Biological Sciences 361:319–334.

Kruuk, L. E. B., J. Merilä, and B. Sheldon. 2001. Phenotypic selection on a heritable size trait revisited. American Naturalist 158:557–571.

Lande, R. 2007. Expected relative fitness and the adaptive topography of fluctuating selection. Evolution 61:1835–1846.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

McLain, D. K. 1992. Population density and the intensity of sexual selection on body length in spatially or temporally restricted natural populations of a seed bug. Behavioral Ecology and Sociobiology 30:347–356.

Milner, J. M., S. D. Albon, A. W. Illius, J. M. Pemberton, and T. H. Clutton-Brock. 1999. Repeated selection of morphometric traits in the Soay sheep on St Kilda. Journal of Animal Ecology 68:472–488.

Mitchell-Olds, T., and R. G. Shaw. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. Evolution 41:1149–1161.

Moore, A. J. 1990. The evolution of sexual dimorphism by sexual selection: the separate effects of intrasexual selection and intersexual selection. Evolution 44:315–331.

Nishida, T. 1994. Spatio-temporal variation of natural and sexual selection in breeding populations of the coreid bug, *Colpula lativentris* (Heteroptera: Coreidae). Researches on Population Ecology 36:209–218.

Peres-Neto, P. R., and D. A. Jackson. 2001. How well do multivariate data sets match? the advantages of a Procrustean superimposition approach over the Mantel test. Oecologia (Berlin) 129:169–178.

Phillips, P. C., and S. J. Arnold. 1989. Visualizing multivariate selection. Evolution 43:1209–1222.

Preziosi, R. F., and D. J. Fairbairn. 2000. Lifetime selection on adult body size and components of body size in a water strider: opposing selection and maintenance of sexual size dimorphism. Evolution 54:558–566.

Punzalan, D. 2007. Evolution of sexual color pattern dimorphism in the ambush bug *Phymata americana*. PhD thesis. University of Toronto.

Punzalan, D., M. Cooray, F. H. Rodd, and L. Rowe. 2008*a*. Condition dependence of sexually dimorphic coloration and longevity in the ambush bug *Phymata americana*. Journal of Evolutionary Biology 21:1297–1306.

Punzalan, D., F. H. Rodd, and L. Rowe. 2008*b*. Sexual selection mediated by the thermoregulatory effects of male color pattern in the ambush bug *Phymata americana*. Proceedings of the Royal Society B: Biological Sciences 275:483–492.

———. 2008*c*. Sexual selection on sexually dimorphic traits in the ambush bug *Phymata americana*. Behavioral Ecology 19:860–870.

Reimchen, T. E., and P. Nosil. 2002. Temporal variation in divergent selection on spine number in threespine stickleback. Evolution 56:2472–2483.

Rundle, H. D., S. F. Chenoweth, and M. W. Blows. 2008. Comparing complex fitness surfaces: among-population variation in mutual sexual selection in *Drosophila serrata*. American Naturalist 171:443–454.

Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. Evolution 45:849–861.

Schluter, D., and D. Nychka. 1994. Exploring fitness surfaces. American Naturalist 143:597–616.

Schluter, D., T. D. Price, and L. Rowe. 1991. Conflicting selection pressures and life history trade-offs. Proceedings of the Royal Society B: Biological Sciences 246:11–14.

Simpson, G. G. 1953. The major features of evolution. Columbia University Press, New York.

Stinchcombe, J. R., A. F. Agrawal, P. Hohenlohe, S. J. Arnold, and M. W. Blows. 2008. Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? Evolution 62:2435–2440.

Sutherland, W. J. 1985. Chance can produce a sex difference in mating success and explain Bateman's data. Animal Behaviour 33:1349–1352.

Svensson, E. I., and B. Sinervo. 2004. Spatial scale and temporal component of selection in side-blotched lizards. American Naturalist 163:726–734.

Turelli, M. 1988. Phenotypic evolution, constant covariances, and the maintenance of additive variance. Evolution 42:1342–1347.

Turelli, M., and N. H. Barton. 2004. Polygenic variation maintained by balancing selection: pleiotropy, sex-dependent allelic effects and G × E interactions. Genetics 166:1053–1079.

Twiss, S. D., C. Thomas, V. Poland, J. A. Graves, and P. Pomeroy. 2007. The impact of climatic variation on the opportunity for sexual selection. Biology Letters 3:12–15.

Weis, A. E., W. G. Abrahamson, and M. C. Andersen. 1992. Variable selection on *Eurosta*'s gall size. I. The extent and nature of variation in phenotypic selection. Evolution 46:1674–1697.

Whitlock, M. C. 1997. Founder effects and peak shifts without genetic drift: adaptive peak shifts occur easily when environments fluctuate slightly. Evolution 51:1044–1048.

Wright, S. 1932. The roles of mutation, inbreeding, cross-breeding, and selection in evolution. Proceedings of the Sixth International Congress of Genetics 1:356–366.

Associate Editor: Allen J. Moore
Editor: Mark A. McPeek