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Contemporary sexual selection on sexually dimorphic traits in the ambush bug *Phymata americana*

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Sexual selection is a potent evolutionary force often invoked to explain observed cases of sexual dimorphism. However, evidence of this process operating on existing phenotypic variation is limited. We investigated whether sexual selection could account for sexual dimorphism in size and color pattern in the ambush bug *Phymata americana.* We considered the alternative hypothesis that dimorphism merely reflects sex differences in habitat use but found no evidence of sex differences in microhabitat during 2 sampling periods in the wild. Although the form of sexual (phenotypic) selection on male lateral color pattern varied between samples, selection consistently favored lateral coloration in males but not size. For females, weight was a consistent predictor of mating status in both the early and the late season. We performed 2 separate laboratory studies to investigate potential proximate mechanisms of sexual selection that might account for the field data. Although we found that male weight predicted male success in direct male–male competition and male courtship intensity predicted success in male–female interactions, we did not detect any role of male color pattern in either laboratory study. These data suggest that visual signaling is unlikely to play a role in the evolution of color pattern dimorphism in this species. Consistent with the field data, our laboratory results also found that female weight predicted the probability of copulation, possibly indicating that female receptivity coincides with female reproductive cycle (i.e., egg maturation). *Key words:* color pattern, phenotypic selection, sexual dimorphism, sexual selection. *[Behav Ecol 19:860–870 (2008)]*

Striking examples of the power of sexual selection for driving phenotypic change are evident in species where the sexes differ dramatically in phenotype (i.e., sexual dimorphism). Although the evolution and maintenance of sexual dimorphism is often attributed to sexual selection, there may be alternative selective (e.g., Baker and Parker 1979; Slatkin 1984; Sherratt and Forbes 2001) and historical explanations (e.g., Fairbairn 1997; Badyaev and Martin 2000). Therefore, it is necessary to determine to what extent current selection acts to promote/maintain observed cases of dimorphism.

In the current study, we investigated the potential role of current sexual selection for shaping sexual dimorphism in the ambush bug *Phymata americana* (Heteroptera: Phymatidae). This species exhibits sexual dimorphism with respect to several metric traits, including size and color pattern (Melin 1930; Kormilev 1960). Whereas sexual size dimorphism is somewhat common in insects, dichromatism is less common and is, in fact, particularly rare in the Heteroptera (Scudder GGE, personal communication). Dichromatism in *P. americana* is puzzling because color pattern in phymatids has typically been considered an adaptation to facilitate crypsis of these sit-and-wait predators in their microhabitat (Dodson and Marshall 1984). Yet, color pattern in this species exhibits the hallmarks of sexual selection—dark coloration is expressed only in the adult stage and some components of color are expressed only in males. A plausible alternative explanation for the observed color pattern dimorphism could be that sexual dimorphism is merely the result of sex-specific viability selection where adult males and females maximize crypsis on different backgrounds (e.g., Jormalainen and Tuomi 1989; Calver and Bradley 1991). A previous study suggested that male and female *P. americana* exhibited different microhabitat preferences in a controlled laboratory setting with various alternative substrates (Greco et al. 1995). However, the extent to which the sexes might differ in their use of microhabitat in natural conditions has not been tested.

To test the habitat hypothesis, we compared patterns of microhabitat use by male and female *P. americana.* In addition, we investigated an alternative hypothesis: whether sexual dimorphism in *P. americana* is accounted for by sexual selection. To test the sexual selection hypothesis, we evaluated the extent to which measures of body size and color pattern predicted mating status in the wild. These studies were performed for 2 sampling dates during the mating season in the same year to determine whether patterns of selection and habitat use were consistent across potentially different ecological conditions. We also conducted 2 separate laboratory studies investigating the potential role of 2 common mechanisms of sexual selection: direct male–male competition (i.e., intrasexual selection) and male–female interactions (i.e., intersexual selection) in exerting sexual selection on size and color pattern.

MATERIALS AND METHODS

Study organism

The source population for all field sampling and collection was a wild population of *P. americana* located in an expanse of old fields at the Koffler Scientific Reserve at Joker's Hill, King, Ontario, Canada (44°03′N, 79°29′W). Ambush bugs are predaceous, sit-and-wait predators that typically occur on inflorescences, where they prey upon various flower-visiting insects. The mating period of *P. americana* typically occurs between the end of July and mid-September, during which their abundance and distribution is likely to change throughout the

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season (Balduf 1939, 1941). Female *P. americana* are relatively sedentary, spending most of their time motionless, apparently lying in wait of potential prey (Mason 1977, 1986). Males, on the other hand, actively search for females and, on finding a female, position themselves on the dorsal surface of the female. From this coupled position atop of the females, males engage in a suite of behaviors indicative of courtship (see Supplementary material, S1). Although actual copulation occurs from a lateroventral position, coupling probably also facilitates mate guarding as coupled males may exclude other males from gaining access to the female (Punzalan D, personal observation).

Traits measured

We focused our analyses primarily on sexually dimorphic body size and coloration. We took 2 measures of size for both sexes: pronotum width and live weight. Pronotum width was measured across the narrowest point between anterior and posterior tubercules of the thorax and is a reliable measure of overall adult body size (Mason 1973). Weight is a measure of size that, though correlated with pronotum width, also may reflect variance in current state or condition; the use of size-corrected (i.e., residual) weight is conventionally used as an index of body condition (Jakob et al. 1996; Kotiaho 1999). Because condition is associated with overall "individual quality" and often explains a large proportion of variation in fitness, the inclusion of weight in the analyses was meant to (at least partially) statistically control for the effects of condition on mating success. Furthermore, weight varies considerably in females according to current reproductive state; female weight is correlated with the number of chorionated eggs in the female reproductive tract (Punzalan 2007). Therefore, including female weight in the analyses was meant to also account for individual variation in egg development. Two aspects of color pattern were measured. For both males and females, we measured the total area of coloration visible from the dorsal aspect (hereafter referred to as "dorsal coloration") (Figure 1). Subsequently, we measured (from digital photographs of live individuals against a color standard) the total visible area of coloration on the lateral surface of the thorax (hereafter referred to as "lateral coloration") in males (females do not express lateral coloration, Figure 1). Pronotum width and color pattern traits were measured from digital photographs using Scion® Image software (http://www.scioncorp.com). Detailed descriptions of the methods used to obtain digital photographs are available in the Supplementary material (S2). Individuals were weighed to the nearest 0.01 mg with an electronic balance. Prior to analyses, color pattern area was square root transformed and weight was cube root transformed to account for scaling with the linear trait (i.e., pronotum width). Morphological traits were $\log_e$ transformed prior to analyses. All traits (morphological and male courtship intensity) were standardized to mean $= 0$ and standard deviation $= 1$ prior to regression analyses. Unstandardized values were used to calculate phenotypic covariances among traits (Table 1).

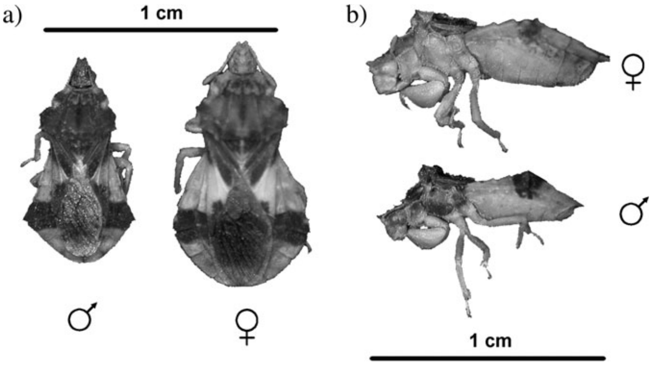

Figure 1
Dorsal (a) and lateral (b) view of sexual dimorphism in size and coloration in *Phymata americana*.

Part 1: sexual selection and habitat use in wild populations

To document mating patterns at different times of the season, we conducted 2 cross-sectional analyses on 19 August (early season) and 7 September (late season) 2002. We standardized sampling effort by collecting all ambush bugs encountered along a ($\sim$) 650-m path during a 4-h period (ca., 1100 to 1500 h). We recorded the sex, mating status of each individual ("single" or "coupled"), and species of plant/inflorescence on which each occurred. We did not differentiate between insects that were coupled or "in copulo" in the field, but coupling success is highly correlated with copulatory success in the laboratory (see Results, Part 3: intersexual selection), suggesting that coupling success is a reasonable surrogate measure of mating success. Copulation often lasts for more than an hour, whereas pre- and/or postcopulatory coupling lasts for several hours and sometimes for more than a day (Punzalan D, unpublished data). Insects were transported live to the laboratory where they were weighed to the nearest 0.01 mg and photographed (see Measuring color pattern traits from digital photographs, Supplementary material, S2). Insects were released at the original collection site 24 h later.

Evaluating sex differences in habitat use

For each sampling date, we assessed whether the sexes used different microhabitats with a goodness of fit test assessing the association between sex and plant species. Bugs were collected from several plant species, including *Daucus carota*, *Solidago* spp., *Achillea millefolium*, *Chrysanthemum leucanthemum*, *Cirsium arvense*, and *Eupatorium maculatum*, though bugs were most frequently ($>95\%$ of all individuals in both samples) encountered on the former 2. Thus, for the analyses, the latter 4 species were lumped into a single (other) category.

Estimating sexual selection in the wild

We estimated phenotypic selection on 4 male and 3 female traits using multiple regressions for each sex separately and for each sampling date. Standardized linear selection gradients (β) were estimated using multiple linear regression of coupling success on the measured traits. Linear selection gradients are measures of direct selection, that is, the effect of a particular trait on relative fitness after statistically controlling for the effects of other measured traits. We also estimated standardized nonlinear selection gradients (γ) from a separate multiple quadratic regression including the linear terms (Lande and Arnold 1983). The nonlinear selection gradients derived from the quadratic regression approximates stabilizing/disruptive selection on individual traits as well as selection acting on linear combinations of traits (i.e., correlational selection gradients). Nonlinear selection may, however, be underestimated unless canonical analysis is performed to identify the major axes of nonlinear selection (Blows and Brooks 2003). We performed the canonical analysis to determine the major axes (m_n) of nonlinear selection. Individual scores (i.e., composite traits) on the new canonical axes were calculated and subjected to 2 separate (the linear and the quadratic) multiple regressions to estimate linear (θ) and nonlinear (λ) coefficients in canonical space (Phillips and Arnold 1989; Blows and Brooks 2003). This procedure essentially provides a means to estimate nonlinear selection with more statistical power.

Table 1

Measured phenotypic traits of male and female *Phymata americana* collected on 2 sampling dates in 2002

	Sampling date #1 ($n = 232$)						Sampling date #2 ($n = 47^a$)					
			Covariances						Covariances			
Males	Mean (SD)	CV	PN	WT	DO	LA	Mean (SD)	CV	PN	WT	DO	LA
PN	2.85 (0.16)	0.057					2.88 (0.14)	0.050				
WT	18.6 (3.2)	0.172	0.620				18.2 (2.3)	0.127	0.695			
DO	13.30 (4.54)	0.215	0.301	0.340			10.01 (2.06)	0.206	0.190	0.290		
LA	10.31 (2.86)	0.441	0.394	0.499	0.562		5.55 (2.32)	0.419	0.155	0.153	0.503	

	Sampling date #1 ($n = 204^b$)					Sampling date #2 ($n = 55$)				
			Covariances					Covariances		
Females	Mean (SD)	CV	PN	WT	DO	Mean (SD)	CV	PN	WT	DO
PN	3.14 (0.20)	0.062				3.19 (0.19)	0.058			
WT	33.4 (8.1)	0.242	0.604			39.7 (7.2)	0.181	0.735		
DO	10.34 (3.46)	0.334	0.371	0.525		4.96 (2.73)	0.550	0.200	0.388	

Traits are pronotum width (PN) in millimeters, weight (WT) in milligrams, and dorsal (DO) and lateral (LA) color patterns in square millimeters. Reported are trait means, standard deviation (SD), coefficient of phenotypic variation (CV), and phenotypic covariances among trait in the early- and late-season samples. Phenotypic covariances are for (unstandardized) traits after transformation.

[a] Sample size for LA = 46.

[b] Sample size for PN = 203 and DO = 201.

Due to the binary nature of our measure of mating success, significance testing for selection coefficients was performed using multiple logistic regression (Janzen and Stern 1998).

Although color standards were included in digital photographs to correct for potential between-sample differences in lighting used during photography, inspection of the images (i.e., comparing the scores for color standards measured in both samples) showed large qualitative differences that could not be corrected. As a result, we did not make quantitative comparisons of absolute (i.e., mean) color pattern area between sampling dates and instead restricted our analyses to comparisons of phenotypic selection coefficients specific to each sample. These differences in mean color pattern, however, do not affect comparison of selection coefficients because our estimates of selection were performed on traits standardized to a mean = 0 and standard deviation = 1; estimates of selection gradients are reported in the currency of standard deviations and, thus, directly comparable across samples (Lande and Arnold 1983; Arnold and Wade 1984a, 1984b).

Comparing patterns of selection between samples

In addition to reporting selection coefficients for each sex and sample, we also tested for differences in the strength/form of selection observed in each sample. To test for between-sample differences (separately for each sex) in the strength of linear selection, we used logistic regression to estimate the relationship between coupling success (response variable) and a number of predictor variables, including each measured trait, a categorical variable representing sampling date (early vs. late season), and the interaction terms between sampling date and each trait. This was performed separately for each sex using data pooled across both seasons but with traits standardized and relative fitness calculated according to sample-specific means/variances. Significant interaction terms were interpreted as evidence of variation across samples in the strength of linear selection. To test for differences in nonlinear selection across samples, we used a combination of sequential model building and partial *F*-tests comparing model fit when interaction terms between a categorical variable (i.e., sample) and continuous variables (i.e., traits) are added/removed (described in Draper and John 1988; Chenoweth and Blows 2005).

Size-assortative mating

We inspected our data for any evidence of size-assortative mating, a type of nonrandom mating and a phenomenon commonly observed in insects (reviews in Crespi 1989; Arnqvist et al. 1996). We calculated the Pearson's product moment correlation coefficient (Bonferroni corrected and uncorrected) for all 4 pairwise combinations between the 2 measures (pronotum width and weight) of male and female size.

Part 2: intrasexual selection

We examined the role of male size and color pattern in determining copulatory success in the presence of immediate male–male competition. We simulated a situation in the laboratory where a dyad of males was allowed equal opportunity to compete for access to a single female. Although this method allows for both intra- and intersexual mechanisms to operate simultaneously, females do not appear to actively approach (i.e., "choose" between) males. Instead, males scramble up/across the substrate and mount the female (i.e., coupling, see Supplementary material, S1), from which position they perform (putative) courtship behaviors (described in Supplementary material, S1) while resisting displacement by other males. Thus, we considered copulatory success in this experiment to primarily reflect variance in the ability of males to gain/retain access to females; this assumes no selection on male courtship, which was measured in a separate experiment (Part 3: intersexual selection).

A total of 96 males and 48 females were individually collected from a field site (18 August 2005) and maintained overnight under laboratory conditions. Bugs were individually housed in (5 mL) glass shell vials prior to all trials. All trials were performed at 27 °C (±3 °C) under 14:10 light:dark full-spectrum illumination. On the following day, at around 1100 h, 48 females were individually placed in a clean vial containing a strip of cardboard substrate for a perch. An hour later, a pair of randomly selected males were simultaneously introduced into each vial. Subsequently, each vial was checked at 20-min intervals until a male had achieved a successful copulation and the males and females were immediately separated and individually frozen in 0.5 mL centrifuge tubes at –20 °C. If 4 h had elapsed without copulation, we defined "success" according to which male was successfully coupled with the female at the end of the trial and the males and females were separated and frozen. Two weeks later, the insects were weighed and photographed. To assess the importance of measured traits in predicting success in direct male–male competition, we first calculated the differences between paired males (within each trial) for each trait as follows:

$$\Delta = (\text{trait value for male A}) - (\text{trait value for male B}).$$

In each trial, the identity of males (A or B) was assigned randomly and Δ was calculated for pronotum width (cube root transformed), weight, and (square root transformed) dorsal and lateral color patterns. We then defined an arbitrary, binary response variable (1 = male A was successful and 0 = male A was unsuccessful) according to our criteria for success, as defined previously. Mutiple logistic regression was used to estimate the extent to which the response variable (the success of male A) was predicted by differences (Δ) in male body size, weight, and dorsal and lateral color patterns after standardization (such that Δ for each trait had a mean of zero and unit variance).

Part 3: intersexual selection

To examine the role of possible intersexual mechanisms in determining copulatory success, we used a no-choice (i.e., a single male and single female) method to exclude male–male competition. Males and females were collected from the field (between 15 and 28 August 2003) and housed in the laboratory overnight (conditions are the same as in Part 2: intrasexual selection). Individuals were weighed and photographed the following morning, approximately 2 h prior to trials. Females were allowed to acclimatize in their own cage for approximately 30 min prior to the introduction of a randomly chosen male. In 4 cases, females had oviposited the evening before the trial and the number of ova deposited was recorded. Males were introduced by gently dropping the cardboard substrate (with male) into the cage containing the female. Recording of behaviors commenced when the male made first physical contact with the female. We measured all mating behaviors and events (described in Supplementary material, S1) occurring until 5 min after copulation (for "successful" trials) or until the trial was ended (and classified as a "failed" trial) according to any of the following criteria: 1) if 1 h had passed without achieving copulation, 2) if males failed to make a copulation attempt for 20 consecutive minutes, or 3) if 3 consecutive uncoupling events were observed without a copulation attempt. We distinguished among these 3 causes of failure as each could represent mating biases exerted by either sex and/or lack of sexual receptivity. The first criterion possibly reflects females failing to accept male mating attempts while the latter 2 criteria may reflect male reluctance to mate. Eighty-five mating trials were observed. For a subset (61) of these trials, we were able to record whether copulating pairs were remained in copulo after 1 h; variation in the duration of copulation is one potential means for generating variance in male fitness, assuming that the amount of sperm or seminal products increases proportionally with copulation duration (e.g., Parker and Simmons 1994; Arnqvist and Danielsson 1999). Behaviors were recorded using an event recorder (The Observer©, Noldus 1991). After mating trials, bugs were individually stored in a 0.5-mL centrifuge tube and frozen at –20 °C for approximately 4 weeks before mounting males on entomological pins.

We used a multiple logistic regression to estimate the combined effects of 5 male morphological traits (pronotum width, antennae length, weight, dorsal, and lateral coloration), male courtship intensity (described earlier in Traits measured), 3 female traits (pronotum width, weight, and dorsal), and 1 interaction term (male pronotum width × female pronotum width) on the probability of copulation. The interaction term was included to account for the possibility of size-assortative mating based on the mechanical constraints imposed by body size (e.g., similar-sized bugs may be more efficient in achieving copulation than pairs of dissimilar size). Similarly, we performed a logistic regression on the same variables to assess which traits predicted copulation duration (i.e., the probability of a pair remaining in copulo after 1 h).

Statistical analyses were performed with Systat® v. 10.0 except for the Principal components analysis which was performed using JMP® v. 4.0.3 (SAS Institute, NC) and the canonical analyses, for which we used the symmetric eigenanalysis function in Poptools 2.6.2 (available at: http://www.cse.csiro.au/poptools).

RESULTS

Part 1: sexual selection and habitat use in wild populations

Habitat use

In the early-season sample, *P. americana* occurred primarily on *D. carota* and, to a lesser extent, on several species of composite flowers including *Solidago* spp.

The sexes showed no differences in their occurrence on different plant species ($\chi^2 = 1.243$, degrees of freedom [df] = 2, $P = 0.537$). In the late-season sample, *Solidago* spp. was the most abundant flowering plant and virtually the only one used by *P. americana* (102 of 104 occurrences). Clearly, the sexes did not differ in the plant species used in either sample.

Sexual selection

For males in the early-season sample, lateral coloration was subject to marginally nonsignificant ($\gamma = -0.408$, $P = 0.067$; Table 2) negative nonlinear selection, suggesting stabilizing selection on lateral darkness. This finding was also supported by the canonical analysis, which revealed significant convex selection on m_4 (early season: $\lambda = -0.582$, $P = 0.025$), an axis where, for a given pronotum width, lateral coloration had the highest, positive loading (Table 3). In the late-season sample, however, male lateral coloration was subject to significant positive directional selection—that is, males with relatively dark lateral coloration had higher mating success (Table 2). Though the samples appeared to differ qualitatively in terms of the strength of linear selection, we detected a consistent trend of overall positive selection on lateral coloration across samples (Wald's $\chi^2 = 4.43$, df = 1, $P = 0.035$; Table 4) and a marginally nonsignificant interaction between sampling date and selection on male lateral coloration (Wald's $\chi^2 = 3.43$, df = 1, $P = 0.064$; Figure 2). We detected no differences across sampling dates in quadratic ($F_{4,259} = 0.495$, $P = 0.740$) or correlational selection on measured male traits ($F_{6,247} = 1.465$, $P = 0.191$).

For females, weight was the only predictor of mating status in both early and late season (Table 2)—relatively heavy females were more likely to be found mating. Positive linear selection on female weight was consistently strong across samples (Wald's $\chi^2 = 12.35$, df = 1, $P = 0.047$; Table 5, Figure 3). We did not detect any differences across samples in quadratic ($F_{3,242} = 0.584$, $P = 0.626$) or correlational selection ($F_{3,236} = 0.394$, $P = 0.758$) on female traits, though canonical analysis revealed significant linear selection acting on axis m_1 in the early-season sample ($\theta = 0.398$, $P < 0.001$; Table 3) suggesting

Table 2

Phenotypic selection coefficients estimating sexual selection on pronotum width (PN), weight (WT), dorsal (DO), and lateral (LA) coloration in wild *Phymata americana* on 2 sampling dates in 2002

	Sampling date #1 ($n = 232$)					Sampling date #2 ($n = 46$)				
		γ					γ			
Males	β	PN	WT	DO	LA	β	PN	WT	DO	LA
PN	−0.111	−0.142				−0.218	0.872			
WT	0.227	0.247	−0.166			0.171	−0.372	0.259		
DO	0.097	0.004	−0.156	0.003		−0.256	−0.290	−0.270	1.073	
LA	0.074	0.064	0.168	0.162	−0.407[a]	0.538*	0.049	−0.225	−0.512	0.270
	$I = 1.983$, $\rho = 0.34$					$I = 1.480$, $\rho = 0.41$				

	Sampling date #1 ($n = 201$)				Sampling date #2 ($n = 55$)			
		γ				γ		
Females	β	PN	WT	DO	β	PN	WT	DO
PN	0.074	−0.039			−0.085	−0.297		
WT	0.463*	0.161	0.061		0.537*	0.582	−0.051	
DO	−0.005	0.005	−0.084	0.043	−0.014	0.098	−0.275	0.253
	$I = 1.590$, $\rho = 0.39$				$I = 1.649$, $\rho = 0.36$			

Sex-specific estimates of variance in relative fitness (I), linear selection gradients (β), and nonlinear selection gradients (γ) are reported. The proportion of individuals found mating is denoted as ρ. Significant values ($P < 0.05$, logistic regression) are denoted by asterisks.

[a] Notably, one additional coefficient was very close to statistical significance ($P = 0.053$).

selection favoring proportionally heavy females for any given pronotum width.

Assortative mating

In the early-season sample, there was weak evidence of assortative mating; the strongest correlation was observed between male pronotum width and female pronotum width ($r = 0.208$, $n = 79$), but this was marginally nonsignificant (uncorrected $P = 0.065$, Bonferroni-corrected $P = 0.391$). The correlations among the other size measures in the early-season sample ranged between $r = 0.014$ and $r = 0.129$ ($n = 79$) and were not statistically significant (uncorrected $P > 0.263$ for all comparisons). Similarly, in the late-season sample, there was a weak positive relationship among male and female size measures (ranging from $r = 0.160$ to $r = 0.364$, $n = 18$), but none of these were statistically significant (uncorrected $P > 0.130$ for all pairwise comparisons). Therefore, we observed only a weak trend toward size-assortative mating in the 2 samples.

Part 2: intrasexual selection

Of the 48 trials observed, all resulted in a successful copulation except for one (98%); in this instance, one male was coupled

Table 3

Estimated linear (θ) and nonlinear (λ) selection gradients for wild *Phymata americana* in 2002 after canonical rotation of the major axes (m) of nonlinear selection (calculated from Table 1)

	Sampling date #1						Sampling date #2					
			Trait loadings						Trait loadings			
Males	θ	λ	PN	WT	DO	LA	θ	λ	PN	WT	DO	LA
m_1	0.052	0.174	0.539	0.670	−0.499	0.105	−0.306	1.460	−0.449	0.026	0.812	−0.373
m_2	0.107	0.050	0.408	0.198	0.792	0.408	0.394	0.972	0.751	−0.521	0.399	−0.072
m_3	0.223	−0.354	−0.711	0.475	−0.020	0.518	0.336	0.336	−0.396	−0.575	0.123	0.705
m_4	0.122	−0.582*	−0.196	0.535	0.350	−0.744	0.265	−0.294	0.278	0.630	0.408	0.599

	Sampling date #1					Sampling date #2				
			Trait loadings					Trait loadings		
Females	θ	λ	PN	WT	DO	θ	λ	PN	WT	DO
m_1	0.398*	0.206	0.499	0.776	−0.385	0.350	0.524	0.433	0.706	−0.560
m_2	0.102	0.030	0.434	0.161	0.886	0.114	0.208	0.517	0.315	0.796
m_3	−0.226	−0.172	0.750	−0.610	−0.256	−0.400	−0.828	0.739	−0.634	−0.229

Also reported are the trait loadings for pronotum width (PN), weight (WT), dorsal (DO), and lateral (LA) coloration on each canonical axis. Significant values ($P < 0.05$, logistic regression) are denoted by asterisks.

Table 4

The effects of sampling date on the strength and form of linear phenotypic selection on male *Phymata americana* in 2002

Variable	α	SE	Wald's χ^2	*P* value
Sampling date	−0.017	0.190	0.01	0.927
Male PN	−0.263	0.253	1.08	0.299
Male WT	0.271	0.269	1.01	0.314
Male DO	0.001	0.292	0.00	0.998
Male LA	0.591	0.281	4.43	0.035*
Sampling date × male PN	0.069	0.253	0.07	0.785
Sampling date × male WT	0.063	0.269	0.06	0.815
Sampling date × male DO	0.423	0.292	2.10	0.147
Sampling date × LA	−0.520	0.281	3.43	0.064

Full model log likelihood = 16.825, df = 9, $P = 0.052$, rho squared = 0.047. Reported are the logistic multiple regression estimates (α) ± standard error (SE) of the effects of sampling date (dummy variable) and measured traits: pronotum width (PN), weight (WT), dorsal (DO), and lateral (LA) coloration for predicting coupling success in males. *P* values <0.05 denoted by asterisks. ($n = 277$).

Table 5

The effects of sampling date on the strength and form of linear phenotypic selection on female *Phymata americana* in 2002

Variable	α	SE	Wald's χ^2	*P* value
Sampling date	−0.049	0.350	0.02	0.889
Female PN	−0.145	0.434	0.00	0.738
Female WT	1.008	0.507	12.35	0.047*
Female DO	−0.029	0.342	0.05	0.933
Sampling date × female PN	0.318	0.480	0.44	0.507
Sampling date × female WT	−0.024	0.567	0.00	0.966
Sampling date × female DO	0.152	0.425	0.13	0.721

Full model log likelihood = 46.982, df = 7, $P < 0.001$, rho squared = 0.138. Reported are the logistic multiple regression estimates (α) ± standard error (SE) of the effects of sampling date (dummy variable) and measured traits: pronotum width (PN), weight (WT), and dorsal (DO) coloration for predicting coupling success in females. *P* values <0.05 denoted by asterisks. ($n = 256$).

with the female while the other male was located away from the pair. The median time from the beginning of the trial until copulation was 260 min. Of the 4 measured male traits, only the difference in male weight predicted success in direct male–male competition ($P = 0.042$, Table 6); relatively heavy males had an advantage in direct male–male competition.

Part 3: intersexual selection

In the majority of trials (70 of 85, 82%), pairs mated successfully. Median time from the beginning of the trial until copulation was 288 s. Copulations occurred on both sides of the female with similar frequency, 37 from the left side and 33 from the right side. Male courtship, female weight, and the body size interaction term all had significant effects on (i.e., were positively related to) the probability of successful copulation (Table 7). Of the 15 unsuccessful matings, 4 failed according to Criterion 1 (females failing to accept male attempts), 3 failed according to Criterion 2 (males failing to make an attempt), and 8 failed according to Criterion 3 (males uncoupling). Of the mating pairs monitored, only 18 of 61 (30%) remained in copulo after 1 h. Only male lateral coloration predicted copulation duration; male lateral coloration was negatively related to the probability of remaining in copulo (Table 8).

DISCUSSION

We investigated the extent to which size and color dimorphism in *P. americana* can be accounted for by sex differences in habitat use or by contemporary sexual selection pressures. Our major findings were as follows: 1) no evidence of sex differences in habitat use, 2) males with relatively dark lateral color pattern had higher mating success in the field but this trait did not predict success in laboratory experiments investigating direct male–male competition and female preference,

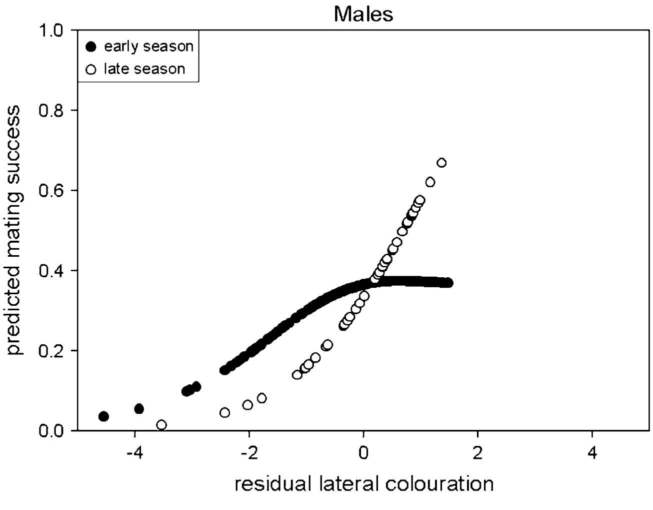

Figure 2
Phenotypic selection on male lateral color pattern in *Phymata americana* in 2002. Cubic splines depict the values (Yhats) that estimate the relationship between standardized area of male lateral coloration (after regression on pronotum width) and mating success in the early-season sample (filled circles) and late-season sample (open circles). Cubic splines were calculated using FORTRAN (Schluter 1988) with a smoothing parameter λ = 1.

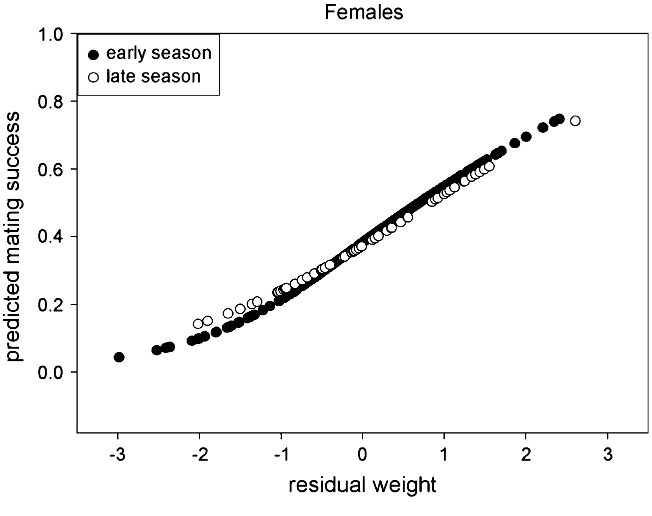

Figure 3
Weight as a predictor of female mating status in *Phymata americana* in 2002. Cubic splines depict the values (Yhats) that estimate the relationship between standardized female weight (after regression on pronotum width) and mating success in the early-season sample (filled circles) and late-season sample (open circles). Cubic splines were calculated using FORTRAN (Schluter 1988) with a smoothing parameter λ = 2.

Table 6

Morphological predictors of male success in direct competition for access to female *Phymata americana* (Part 2, laboratory study)

Variable	α	SE	*t* Ratio	*P* value
(Δ) Male pronotum	−0.623	0.517	−1.205	0.228
(Δ) Male weight	1.098	0.538	2.037	0.042*
(Δ) Male dorsal	0.174	0.403	0.431	0.667
(Δ) Male lateral	0.352	0.412	0.855	0.392

Full model log likelihood = 8.066, df = 4, $P = 0.089$, rho squared = 0.127. Reported are logistic multiple regression estimates (α) ± standard error (SE) of the effects of each independent variable (corresponding to male traits). Independent variables in model represent the trait difference (Δ) between paired males in each trial (i.e., male A − male B). The response variable was coded as: 1 = male A won contest and 0 = male A lost contest (i.e., male B won; see Materials and Methods for explanation). *P* values <0.05 denoted by asterisks ($n = 48$).

3) male weight predicted success in direct male–male competition, 4) female weight predicted mating probability in both field and laboratory studies, and 5) there was only weak evidence of size-assortative mating. These results are discussed in more detail below.

Microhabitat use

One possible cause of sexual dimorphism is ecological differentiation between the sexes (Slatkin 1984). A laboratory study by Greco et al. (1995) presented some evidence indicating that male and female *P. americana* exhibited sex differences in habitat preferences, where females exhibited nonrandom preference in an array of different flower species (preferring *Solidago canadensis* and *D. carota* over *C. arvense*), whereas males were indiscriminate. Our study, however, found no evidence of sex differences in habitat use in the wild in either sampled period. Our study could reflect the fact that, under natural conditions, females can choose the preferred (plant) species, whereas males probably occur on plants where females are most abundant. This is consistent with female fitness being limited by food resource availability (hunting sites and prey), whereas male fitness is primarily limited by the availability of mates (Mason 1986). We should note that our study cannot rule out that males and females use the available plant species in different ways. For example, the sexes may occupy different parts of a plant/inflorescence or assume different postures. Given that the main plant species used differ according to a number of important ecological dimensions (e.g., color, shape, and structure), there is potential for fine-scale differences in microhabitat use. Patterns of habitat use by males and females may also depend critically on what alternative plant species are available. In addition to the observed temporal differences (Part 1: sexual selection and habitat use in wild populations), the distribution and abundance of plant species potentially used by *P. americana* is likely to vary spatially. As a result, any sex differences in preferences for different microhabitats (Greco et al. 1995) may be more or less apparent in different sampled times or populations. Further work is required to address the possibility of sex differences in distribution at finer scales, their potential effects on male and female viability, as well as any interaction with spatiotemporal variation in available plant communities.

Table 7

Morphological and behavioral predictors of copulatory success in *Phymata americana* (Part 3, laboratory study)

Variable	α	SE	*t*	*P* value
Male pronotum	0.148	0.818	0.181	0.856
Male weight	−0.567	0.759	−0.747	0.455
Male dorsal	−0.243	0.982	−0.248	0.804
Male lateral	−1.227	0.962	−1.227	0.202
Male antenna	0.540	0.442	1.221	0.222
Male courtship	1.070	0.441	2.426	0.015*
Female pronotum	−0.738	0.845	−0.873	0.383
Female weight	1.471	0.694	2.120	0.034*
Female dorsal	0.283	0.548	0.515	0.606
Male pronotum × female pronotum	2.035	0.912	2.231	0.026*

Full model log likelihood = 24.915, df =10, $P = 0.006$, rho squared = 0.417. Reported are logistic multiple regression estimates (α) ± standard error (SE) of the effects of morphology and behavior of male–female pairs on the probability of copulation. *P* values <0.05 denoted by asterisks ($n = 78$).

Table 8

Morphological and behavioral predictors of copulation duration in *Phymata americana* (Part 3, laboratory study)

Variable	α	SE	*t*	*P* value
Male pronotum	0.162	0.530	0.306	0.760
Male weight	−0.733	0.598	−1.225	0.220
Male dorsal	0.602	0.670	0.889	0.369
Male lateral	−1.784	0.730	−2.445	0.014*
Male antenna	1.205	0.709	1.700	0.089
Male courtship	−0.529	0.337	−1.569	0.117
Female pronotum	0.550	0.470	1.171	0.242
Female weight	−0.846	0.515	−1.642	0.101
Female dorsal	1.220	0.696	1.752	0.080
Male pronotum × female pronotum	−0.001	0.473	−0.002	0.998

Full model log likelihood = 0.288, df =10, $P = 0.020$, rho squared = 0.288. Reported are logistic multiple regression estimates (α) ± standard error (SE) of the effects of morphology and behavior of male–female pairs on the probability of copulating for 60 min. *P* values <0.05 denoted by asterisks ($n = 60$).

Sexual selection on color pattern

We found that, in our sampled population, male (lateral) color pattern was subject to significant sexual selection—albeit of different form in each sample (discussed below). Male lateral coloration was generally favored, suggesting that this trait is indeed a sexually selected secondary sex character, providing evidence that sexual dimorphism in this population is promoted by current selection pressures. Although this may explain the presence/maintenance of this trait in males, it only provides a partial explanation for sexual dimorphism in lateral coloration. The lack of dark coloration in females is suggestive of lateral coloration being (or having been) selectively disfavored in females. Although direct measures of viability selection on coloration (in either sex) are not currently available, there is some indirect evidence that color pattern is costly in terms of resource allocation. Development of dark color is strongly limited by diet (Punzalan 2007), suggesting that dark pigmentation may be energetically costly to produce. If so, sex differences in color pattern could reflect selection for females to suppress allocation to this trait, whereas in males, the costs of allocating limited resources to color pattern development are offset by the fitness advantages in terms of mating success.

Although our phenotypic selection analyses suggest that some components of sexually dimorphic coloration (lateral pattern) in *P. americana* can be accounted for by contemporary selection pressures, they fail to provide an evolutionary explanation for dimorphism of other components of color pattern; we did not find any evidence of directional selection on dorsal coloration (in either sex) despite its elevated expression in males. It is possible that dimorphism in dorsal coloration reflects genetic constraints if elevated expression in males is caused by a correlated response to selection on male lateral coloration. For example, a common physiological mechanism underlying expression of both components of coloration in males may result in shared, pleiotropic effects on color pattern traits. Consistent with this scenario, dorsal and lateral coloration did indeed exhibit positive phenotypic correlations for field-caught populations (e.g., Table 1, also see Punzalan 2007) and for laboratory-reared populations where diet was manipulated (Punzalan et al. forthcoming).

Alternatively, we may have failed to capture the true (net) fitness surface for dorsal color pattern as the current study only considered selection via mating success. Color pattern may have important viability consequences in terms of conspicuousness/crypsis to visually orienting predators (Endler 1978; Edmunds and Grayson 1991) as well as prey (Grether and Grey 1996); and it seems likely that such selection pressures apply to sit-and-wait predators like *P. americana.* Color pattern may also be subject to viability selection due to the thermal effects of color pattern on survival (e.g., Ottenheim et al. 1999) or fecundity (e.g., Fischer et al. 2003). Future studies elucidating the role of color pattern in mediating various components of viability are required.

Selection on body size and size-assortative mating

In insects, males are typically smaller in size than their female counterparts. One hypothesis put forth to explain this trend is that small size is advantageous in some mating systems, particularly, when male mobility/maneuverability is hindered by large size (reviewed in Blanckenhorn 2000). In contrast, large male size can confer advantages for various aspects of mating success (Andersson 1994). Previous reports on related phymatids (i.e., *Phymata fasciata*, *Phymata wolfii*) suggest a possible advantage afforded by large male size (Dodson and Marshall 1984; McLain and Boromisa 1987). In our study, we did find that males who were relatively heavy for a given body size were more likely to be successful in direct male–male competition in the laboratory. If weight (after accounting for size) is a correlate of male condition, then this could indicate that males who were in good condition experienced a competitive advantage. However, any advantage conferred by large male size was not apparent in terms of mating success in the wild; we detected no (positive or negative) selection on either measure of male size. Thus, our results failed to find evidence that small size is advantageous in terms of sexual selection in this species, though, of course, small male size could still be selectively favored in terms of other, unmeasured components of fitness (e.g., survival).

On the other hand, the observed pattern of sexual size dimorphism could be the result of selection favoring large female size. Whereas large female size is often attributed to fecundity selection, in some species, selection may be partly mediated by males actively preferring to mate with large females (reviewed in Bonduriansky 2001). We found no evidence that female size (i.e., pronotum width) per se predicted female mating status, though our analyses revealed sexual selection favoring a positive correlation between female morphological size and weight in the early season. Consistent with previous findings in related species (Dodson and Marshall 1984; McLain and Boromisa 1987), we also found that weight (after controlling for body size) was a significant predictor of female mating status in the wild. This was corroborated by a no-choice laboratory study that demonstrated that copulatory success was again predicted by female weight (Part 3: intersexual selection). Because residual weight is strongly correlated with the number of mature ova in the female reproductive tract (Punzalan 2007), one possibility is that males exert mate choice favoring more fecund females (or those close to oviposition). Although this hypothesis requires testing, our data did not indicate that female weight elicited a heightened response from males (Supplementary material, S3). A parsimonious explanation for the consistent effect of female weight on mating status is that female receptivity to mating corresponds to egg development/maturation.

Selection on body size can also be manifested through size-assortative mating where larger males, despite not experiencing higher mating rates than small males, have greater reproductive success because they are able to obtain access to larger, more fecund females. Although this phenomenon is common in other insects, the current study revealed only weak evidence of size-assortative coupling in wild populations of *P. americana.* In the laboratory no-choice trials (Part 3: intersexual selection), we found that although the absolute sizes of males or females were not predictors of successful copulation, the relative body size (with respect to one another) was important; pairs of similar size had higher probability of copulating. This could reflect mechanical constraints on copulation. The position from which males and females copulate consists of the male grasping either side of the female thorax and the pair aligning their genital sclerites, followed by intromission (Balduf 1941), and it seems plausible that the efficiency with which pairs achieve copulation is a function of their morphological similarity. Whatever the underlying cause, this size-assortative effect on copulation success could mean that our estimates of mating success based on coupling could underestimate selection on size. However, we should also point out that assortative mating due to such mechanical constraints does not necessarily predict directional selection on size in either sex and, thus, fails to provide a proximate explanation for the observed sexual size dimorphism.

Proximate mechanisms of sexual selection on color pattern

Precopulatory sexual selection is typically attributed to 2 main mechanisms for generating variance in mating success: male–male competition and/or female mate choice. In our laboratory studies, we found evidence of male–male direct competition (i.e., physical interactions) for access to mates. In Part 2: intrasexual selection we saw male–male behaviors that resemble struggling/jockeying for position on the female. The much longer mean time required to achieve a successful copulation in Part 2: intrasexual selection than in Part 3: intersexual selection (where male–male competition was experimentally excluded) is also consistent with direct male–male competition being potentially important in this species. However, we found no evidence that success in direct male–male competition for access to mates was related to male color pattern. Similarly, we found no evidence that selection on lateral coloration (as observed in the wild) was due to female mate choice. Whereas male courtship effort was positively related to copulatory success, none of the male morphological traits were important predictors. Thus, unlike in many other sexually dichromatic insect species (examples in Andersson 1994; Kemp et al. 2005), color pattern in *P. americana* does not appear to play any signaling function in either male–male or male–female interactions.

Curiously, dark male lateral coloration was associated with shorter copulation durations. There are several possible explanations for this observed pattern. One possibility is that females terminate copulation earlier when mating with darker males. However, there was no indication that female behavior resulted in the termination of copulation (i.e., there are no visible behaviors that immediately precede the withdrawal of the male intromittent organ). A second possibility is that male coloration is positively correlated with the rate at which seminal fluids are transferred. The mechanical efficiency of sperm transfer has been reported to correlate with morphology in other species (e.g., body size: Parker and Simmons 2000; Horton et al. 2001), though in this case it is unclear how pigmentation in the thorax could mediate the rate of transfer. A third possibility is that variance in male copulation duration actually reflects age-related differences. Testes size and sperm supply have been shown to decrease with age and/or mating experience in some insects (e.g., Ward and Simmons 1991). Because lateral coloration of *P. americana* darkens with age (Punzalan 2007), it is possible that darker, older males have depleted sperm reserves and, therefore, shorter copulation durations. Finally, it is also possible that variance in male coloration is associated with differences in male mating strategies. Because darker males have higher coupling success (Part 1: sexual selection and habitat use in wild populations) and may be in better condition (Punzalan 2007), dark males may perceive that they have greater future mating opportunities and, as a result, invest less time (and possibly less seminal fluid and sperm) in each copulation. Conversely, relatively pale males may benefit from investing more time and/or sperm in each mating to maximize their mating success. Such patterns of phenotype-dependent mating strategies have been reported in other taxa (e.g., Ward and Simmons 1991; Parker and Simmons 1994, 2000; Rowe and Arnqvist 1996; Arnqvist and Danielsson 1999; Ortigosa and Rowe 2003).

Despite the apparent lack of selection on male color pattern exerted by either direct male–male competition or female mate choice, the pattern of phenotypic selection observed in the wild could be accounted for if variance in male mating success is governed primarily by variation in mate-searching success (which was, for the most part, experimentally excluded in this study). Coupling appears to be a very efficient mode of precopulatory mate guarding in *P. americana*; successfully coupled males are rarely dislodged by competing males (unpublished data), and the majority of males who attempted copulations in Part 3: intersexual selection were ultimately successful (70/74 = 95%). Collectively, these findings are consistent with the notion that male mating success is probably limited most by the ability to find receptive/uncoupled females. This raises the question as to what possible role color pattern could play in determining searching success. A recent study (Punzalan et al. 2008) indicates that color pattern plays an important thermoregulatory role that influences mate-searching success in a manner consistent with the estimates of sexual selection obtained in Part 1: sexual selection and habitat use in wild populations. It is well recognized that selection often acts on different traits at different stages (or episodes) of sexual selection (e.g., Moore 1990; Bonduriansky and Rowe 2003); in this species, we suggest that dark color pattern is the target of sexual selection during mate searching but, on successful location of females, traits other than coloration (i.e., body condition and courtship behavior) determine copulatory success.

Variability in the form and strength of sexual selection

We found that in the early-season sample, lateral coloration experienced convex nonlinear (approximately stabilizing) sexual selection, whereas in the late-season sample, the same trait experienced positive directional selection. There are several possible explanations for this difference; one possibility is that this reflects variable selection pressures on male color pattern associated with changing ecological or demographic conditions. For example, both abundance (density) and sex ratio differed between sampling periods, and both factors are considered to be crucial in determining the dynamics of mating systems because they determine the extent to which males can gain access to females (Emlen and Oring 1977). Somewhat consistent with previous studies that have observed density-dependent sexual selection (e.g., McLain 1982, 1992; Conner 1989; Arnqvist 1992; Carroll and Salamon 1995), directional selection on male *P. americana* was strongest (detectable) only when population density was relatively low. Not surprisingly, the 2 sampling dates also represent considerably different environmental conditions. The late-season sample was conducted in the fall when day length is shorter and temperatures are cooler than in the early season. Because color pattern affects thermoregulatory function and, ultimately, mating success (discussed above), selection on coloration might be expected to vary with seasonal changes in temperature. However, further studies are required to determine whether the observed patterns of selection are truly a reflection of temporal variation in selection corresponding to changing ecological variables.

Alternatively, the difference in pattern of selection also raises some concern over the accuracy of our estimates of selection, in addition to the usual caveats associated with the use of multiple regression to estimate direct selection (discussed in Lande and Arnold 1983; Arnold and Wade 1984a, 1984b; Mitchell-Olds and Shaw 1987). First, our estimate of mating success in this study was limited to cross-sectional samples of mating success. Obviously, mating probability at any given time need not be a reliable estimate of lifetime (longitudinal) mating success. The extent to which cross-sectional and longitudinal estimates of sexual selection in *P. americana* are in agreement is the subject of a current study. Second, our estimate of fitness (mating success) relied on the use of coupling success as a surrogate measure of copulatory success. Although the 2 events are highly correlated in the laboratory (Part 3: intersexual selection), clearly, these are only coarse metrics, several steps removed from a more complete measure of reproductive success (e.g., fertilization). Thus, our analyses should be interpreted as strictly limited to describing precopulatory sexual selection. We should also point out, however, that the inverse relationship between color pattern and copulation duration implies that the advantage of dark males in our cross-sectional estimates in the field might actually be an underestimate. That is, males with darker lateral coloration (who have shorter copulation durations) might be less likely to be found coupled during sampling.

Conclusions

Our studies found no obvious sex differences in microhabitat use and instead suggest that sexual dichromatism in *P. americana* reflects sexual selection. Sexual selection appears to favor dark lateral coloration in males, though directional selection on this trait may only be exerted under certain environmental or ecological circumstances. Although male body condition (weight, after accounting for size) was important in male–male interactions and courtship rate was important in determining the outcome of male–female interactions, color pattern does not appear to serve any signaling function in either male–male or male–female interactions. Although we consistently found that relatively heavy females were more likely to mate, we did not detect patterns of contemporary sexual selection that could account for the observed size dimorphism in *P. americana*. It is possible that sexual size dimorphism is the result of divergent viability selection or merely reflective of sexually size-dimorphic ancestors.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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