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Male mating bias and its potential reproductive consequence in the butterfly *Colias eurytheme*

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Abstract Male mating biases may be a widespread feature of animal mating systems but the phenotypic consequences of these biases are often unclear, especially in species for which the operational sex ratio is strongly male-biased. In *Colias* butterflies, male choice is thought to be one of the factors responsible for maintaining a female-limited genetic color polymorphism, in which female wings appear either yellowish-orange or white (the "alba" variant). Previous studies have indicated that alba females of two montane *Colias* species mate fewer times during their lifetime, possibly as a partial consequence of this bias. Here we report the results of a field study of male mating behavior and female mating biology in *Colias eurytheme*, conducted under conditions of high (summer) and low (spring) population densities. Our data show that despite a substantial male bias in approaching alba vs yellowish-orange phenotypes [ratios of 0.08:1 (spring) and 0.28:1 (summer)], alba females did not contain, on average, fewer or smaller spermatophores. Not one of the 308 sampled females was virgin, but females of both phenotypes accumulated spermatophores with age, and tended to carry fewer, larger spermatophores in spring. These data suggest that significantly fewer (or lighter) spermatophores need not be an obligatory or simple consequence of a strong male bias in butterflies. We discuss these findings in light of the known, thermally and density-dependent complexities of alba reproductive biology and of the *Colias* mating system.

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Introduction

At least since Darwin (1871), biologists have recognized that males and females have different roles, and that members of one sex generally compete more intensely for access to receptive mates. Sex role theory directly attributes this competitive imbalance to the operational sex ratio (OSR; Emlen and Oring 1977; although, see Kokko and Johnstone 2002), which is essentially a measure of the relative number of members of each sex willing (or able) to mate at any given time. The OSR, in turn, is ostensibly determined by levels of parental investment (Trivers 1972) and the potential reproductive rate of each sex (Clutton-Brock and Vincent 1991). Because male parental investment is often relatively low, their rate of reproduction potentially high, and the OSR strongly male-biased, males are characterized as being more sexually competitive and promiscuous, and more likely to exhibit behavioral and/or morphological adaptations such as territoriality, weaponry, and ornamentation. Females, on the other hand, are generally viewed as being less promiscuous and to discriminate more strongly among potential mates. As with many generalizations, however, these "typical" sex roles apply only in the very broadest sense (Houde 2001), with cases of both partial (e.g., Amundsen and Forsgren 2001) and complete (e.g., Simmons 1992) sex role reversal becoming increasingly well documented (Bondurianksy 2001).

Mate choice (or mating bias) is traditionally associated with members of the less sexually competitive sex, which—as per Darwin's (1871) definition—are females. More recently, however, it has become clear that males are often

choosy (Bondurianski 2001), and that such behavior need not be associated with a female-biased OSR and/or complete sex role reversal (e.g., Amundsen and Forsgren 2001). This being the case, an interesting consideration is whether and how such male mating biases exert sexual selection upon female phenotypes (Bondurianski 2001). As classically demonstrated by Bateman (Bateman 1948; also see Arnold 1994; Jones et al. 2002), males and females differ fundamentally in their standardized variance in reproductive success and, hence, in the total intensity of sexual selection that they may possibly experience. Even given that females may (and often do) receive additional direct and/or indirect benefits from mating multiply (e.g., Arnqvist et al. 2004; Bondurianski et al. 2005), male encounters have costs for females (Fowler and Partridge 1989; Kemp and Rutowski 2004), and the optimal female mating rate is almost always expected to be lower than that for males (thus contributing to sexual conflict over mating rate; Arnqvist and Nilsson 2000; Wiklund et al. 2001). These considerations suggest male mating biases as a less potent evolutionary phenomenon (than female biases), particularly in species in which the OSR is male-biased and males typically compete for mating opportunities. Currently, however, there is little direct empirical data regarding the phenotypic outcome of male biases in such systems (refer to the review of Bondurianski 2001).

In this study we investigate the presence and potential selective consequence of male mating bias in the orange sulfur butterfly, *Colias eurytheme*. As with most other members of the genus, *C. eurytheme* exhibits a sex-limited genetic wing-color polymorphism consisting of two female forms: a yellowish or yellowish-orange form and a white or greenish-white "alba" form (Remington 1954). Males of several *Colias* species are known to discriminate against the alba form, and this is matched by lower relative lifetime mating rates in this phenotype (Graham et al. 1980). This mating bias has been proposed as one of the potential sources of selection acting to maintain the polymorphism in these species (Watt 1973; Graham et al. 1980; Gilchrist and Rutowski 1986; Nielsen and Watt 2000). The maintenance of this polymorphism is a complex issue (Nielsen and Watt 1998, 2000), but the inclusion of mate attractiveness implies that male mating biases have significant selective consequences for the female phenotype. This is of interest because *Colias* populations have a typically highly male-based OSR (e.g., Nielsen and Watt 2000) and males compete intensely (via scramble competition) for matings.

Given that male *Colias* do possess a mating bias, alba females may experience a reduction in fecundity due to a suboptimal number or pattern of lifetime encounters with quality mates (Graham et al. 1980; Gilchrist and Rutowski 1986; Nielsen and Watt 1998, 2000). Excluding indirect (genetic) mating benefits, the literature suggests two

potential and nonmutually exclusive ways in which such a bias could reduce alba fitness:

(1) Because alba females will be approached less frequently by males, they may experience greater latencies until their first mating (Nielsen and Watt 2000). Depending upon the duration of this latency, which in turn will probably depend upon population density, alba females could suffer from spending a more sizeable proportion of their adult lifetime in a nonfertile state (Nielsen and Watt 2000).

(2) Male *Colias* donate a single nutritious spermatophore (plus accessory materials) to females during mating (Rutowski and Gilchrist 1986), and the nutrients contained within male butterfly ejaculates have been linked to increased female life span and fecundity (Boggs and Gilbert 1979; Boggs and Watt 1981; Rutowski et al. 1987; Karlsson 1998). Given this form of fitness-enhancing paternal investment, females of the relatively less attractive alba form may suffer from mating fewer times throughout their lifetime and therefore from receiving less male-derived material (Graham et al. 1980). Related to this possibility (but not posed in the literature), the lower approach rates to alba females could see them as being more eager to mate and less choosy on average [see Rutowski (1985) regarding female mate choice in *C. eurytheme*]. If they are more likely to accept smaller or older mates, alba females could suffer from receiving the smaller nuptial investments that these males are known to provide (see, e.g., Rutowski and Gilchrist 1986).

Here we present data on male mating biases and female mating biology (spermatophore frequency and weight) in an agricultural *C. eurytheme* population. Prior studies of the alba polymorphism have used spermatophore counts to infer the presence of sexual selection (Graham et al. 1980; Gilchrist and Rutowski 1986), but no study has yet assessed spermatophore mass. As we outline below, both of these parameters may be relevant to an assessment of sexual selection in *Colias*. However, the use of spermatophore frequency and mass as indicators of male investment is complicated because these parameters are under the influence of both male and female behavior and reproductive physiology, and these processes may vary depending upon female phenotype (see below). More generally, the consequences of male mating biases should also depend upon population density (Nielsen and Watt 1998, 2000). We attempted to address this issue by conducting operations in two separate seasons (spring and summer) that differ markedly in *C. eurytheme* population density (but also in ambient temperature, which may differentially affect mating dynamics between the phenotypes; see below).

Reproductive biology of *Colias* and the alba phenotype

Colias eurytheme is a pierid butterfly with an extensive range across the USA. In south central Arizona, this species occurs in populations centered upon agricultural fields of alfalfa (*Medicago sativa*), which is a larval host. Adults of these multivoltine and continuously breeding populations are on the wing for most of the year (from early March to late November), and the adult generations are indiscrete. Densities can reach extreme levels in localized areas from mid-July to early September (see, e.g., Silberglied and Taylor 1978). The reproductive biology and adult behavior of this species has been extensively studied (Silberglied and Taylor 1978; Boggs and Watt 1981; Rutowski et al. 1981, 1987; Rutowski 1985; Rutowski and Gilchrist 1986; Kemp and Rutowski 2004). As noted earlier, upon mating, the males of this species donate a nutritious spermatophore, which the females erode over time as nutrients are extracted and allocated to eggs and somatic maintenance. Females have a refractory period immediately after mating which is linked to the amount of spermatophore depletion; hence, they are thought to eventually become receptive again to gain a fresh spermatophore (Rutowski et al. 1981). Based upon this model, females accumulate and successively deplete spermatophores throughout their adult lifetime. Smaller males and males that have mated previously or recently produce smaller ejaculates (Rutowski and Gilchrist 1986), and females generally prefer younger mates, perhaps to secure large ejaculates (Rutowski 1985). Data on field-released virgin female *C. eurytheme* suggest they remate once every 4 to 6 days in summer, and realize a lifetime total of up to four matings (Rutowski and Gilchrist 1986).

Features of alba female biology are relevant to this lifetime pattern of mating and resource use and, thus, to the opportunity for sexual selection on this phenotype. Alba females (carriers of at least one dominant *A* allele at a single "alba" locus; Gerould 1923; Remington 1954) appear white because they reappropriate the precursors of pteridine pigments otherwise responsible for yellowish-orange wing coloration (Watt 1973). By reallocating these nitrogenous compounds into alternative metabolic pathways, these individuals may achieve faster juvenile development, increased egg maturation, and lifetime egg production (Watt 1973; Graham et al. 1980; Gilchrist and Rutowski 1986). This intrinsic resource advantage means that alba females should be less reliant on male-derived materials to fuel egg production, perhaps leading to a lower rate of spermatophore erosion and lower obligatory lifetime mating frequency (provided that alba females receive spermatophores that are, on average, as large as those received by their yellowish-orange counterparts). If alba females tend to receive smaller spermatophores (e.g., if they are less choosy due to their lower attractiveness), then they may mate more

often, and their lifetime mating rate may approach that of yellowish-orange females. Alba's resource-based advantage is also most pronounced under cooler conditions (Graham et al. 1980), which means that they should carry over relatively more larval resources for use in egg production at cooler times of the year or in cooler climates. They may also spend more time resting and basking under cooler conditions (Nielsen and Watt 1998). Both of these thermal effects could see alba females erode spermatophores at a lower rate, thus leading to even greater divergence from the mating biology of yellowish-orange females (although other scenarios are possible). We revisit this complex set of issues in the discussion of our findings.

Materials and methods

Model approach experiment

We assessed male approaches to female models at alfalfa fields near Avondale, AZ, on the mornings of August 30 and 31, 2004 (summer) and at a field near Chandler, AZ, on April 26 2005 (spring). The Avondale and Chandler habitats are less than 20 miles apart and almost identical in all respects; we chose different fields in different seasons due to logistical reasons. All work was done with the same dead field-caught females that had been dried for several days in the dorsal "open wing" position. Starting 10:00 h (summer) and 11:00 h (spring), we placed eight yellowish-orange/alba female pairs in the alfalfa field, two heterogeneous pairs at a time, each of which we videotaped for a period of 30 min [thus totaling 8 h (summer) and 4 h (spring) of total "observation" time for models of each phenotype]. Pair members were suspended on fine gauge wire approximately 1 m apart, and 0.4 m above the ground (roughly 50–100 mm above the level of the alfalfa "canopy"). We later tabulated male approaches from the videotapes. Approaches were classified as (a) "contacts," cases in which the male apparently contacted, or otherwise came to within 50 mm, of the model, or (b) "inspections," cases in which the male clearly deviated from his flight path in response to the model but ultimately failed to approach within 50 mm. These classifications are broadly equivalent to the "visits" and "dips" as defined by Graham et al. (1980). Given that our design was perfectly balanced, and that the primary comparison was between models of different phenotypes, we collapsed data across pairs (and the two sampling days in summer) for analysis.

Female mating biology

We haphazardly sampled alba and yellowish-orange phenotype females from cultivated alfalfa fields in Avondale and Chandler

(sample sizes are given in Table 1). Here we sampled on 8 days during the period from 29 August to 7 September 2004 (summer) and on 13 days from 17 April to 25 May 2005 (spring). These two sampling periods were chosen to maximize variation in population density, although spring sampling was initiated at least 5–6 weeks after adults typically first appear in these fields. There were differences in ambient temperature during the sampling periods and in the preceding weeks (the period in which sampled individuals would have developed and spent their precapture adulthood): as a rough guide, the mean ambient temperatures in March (18°C), April (22°C), and May 2005 (28°C) were much lower than those in July (34°C) and August 2004 (33°C) (source: National Climatic Data Center). Sampled females were frozen and later dissected to retrieve spermatophores, which we dried at 60°C for 24 h prior to weighing (to the nearest 1×10^{-5} g) using a Mettler H51AR balance. Female age was classified according to three wing-wear classes, which has been used before in studies of *C. eurytheme* (Rutowski 1985) and of the alba polymorphism (Graham et al. 1980; Gilchrist and Rutowski 1986). We measured forewing length (from apex to insertion, to the nearest 0.1 mm) as a surrogate for body size. Alba phenotypes in our sample did not differ from yellowish-orange phenotypes either in body size (independent t test: spring-$t_{106}=1.42$, $p=0.16$; summer-$t_{198}=0.48$, $p=0.63$) or in wing wear (Mann–Whitney U-test: spring-$U=854$, $p=0.64$; summer-$U=4,671$, $p=0.42$).

Statistical analyses

We used standard statistical procedures throughout except for our analyses of spermatophore frequency and mass, in which we adopted a maximum likelihood-based model fitting approach using the generalized linear/nonlinear modeling function of STATISTICA (version 7.0). This is a "data-based" approach to selecting the model that best accounts for the observed variation in the variable(s) of interest (in this case, spermatophore frequency and mass) and allows one to specify nonnormal distributions for dependent variables. In line with "information theoretic" approaches to the analysis of nonexperimental data (see Burnham and Anderson 2002), we evaluated a model fit using Aikaike's information criterion (AIC). The AIC is equivalent to $-L_q+2q$, where L_q is the maximized log-likelihood, and q is the number of variables in the model. The use of this criterion is an improvement upon simply using the log-likelihood value to compare among candidate models because it adjusts for varying numbers of parameters. An information-theoretic approach is strongly recommended for observational data (as opposed to experimental data) because traditional approaches (e.g., analysis of variance) are often theoretically unjustified and have been shown to perform poorly in simulations [refer to Burnham and Anderson (2002) for an in-depth treatment of this issue].

We selected the best fitting model as that which minimized the AIC (with the caveat that interaction terms were always accompanied by the relevant main effects). Variables initially included in each best subset run are stated in the results. Once the best fitting model was identified, we assessed its overall statistical significance, and the significance of individual model parameters, using log-likelihood and Wald tests, respectively (both of which are evaluated against the chi-squared distribution with degrees of freedom equal to the number of parameters of interest; Agresti 1996). The Wald test is a standard log-likelihood-based test to assess the significance of model parameters (Agresti 1996). We also calculated semipartial r-values [which is a measure of the covariance between one predictor (x_1) and the dependent variable (y) with the effects of other predictors ($x_{1...N}$) removed from y] as estimates of the size and direction of individual effects in multivariable models. In our analysis of spermatophore frequency, in which the dependent variable is a count, we specified an ordinal multinomial distribution; whereas in the analysis of spermatophore mass, we specified a normal distribution (we normalized spermatophore mass using a square-root transformation). Means are accompanied by 95% confidence intervals throughout.

Table 1 Summary of statistics for females, grouped by phenotype, for the spring and summer samples

Phenotype	N	Wing length (mm)	Spermatophores			
			Wing wear	Frequency	Range	Mean weight (g^{-4})
Spring						
Yellowish-orange	87	24.4±0.4	1.44±0.13	1.38±0.12	1–3	8.44±1.12
Alba	21	24.9±0.7	1.33±0.22	1.43±0.27	1–3	9.63±3.08
Subtotal	108	24.5±0.3	1.42±0.11	1.39±0.11	1–3	8.67±1.06
Summer						
Yellowish-orange	100	26.3±0.3	1.68±0.14	1.65±0.14	1–4	6.20±0.71
Alba	100	26.4±0.3	1.58±0.13	1.62±0.14	1–3	7.03±0.79
Subtotal	200	26.3±0.2	1.63±0.10	1.64±0.10	1–4	6.61±0.53

Parameters are quoted with 95% confidence intervals

Results

Model approach experiment

We recorded grand totals of 12 (spring) and 394 (summer) approaches to the female models. Even accounting for the fact that we observed the models for twice as long in summer, these frequencies are indicative of a very large seasonal difference in population density. The inspecting male came to within 50 mm of the model (i.e., a "contact" approach) in 11 (92%) cases in spring and 231 (59%) cases in summer. In spring, 11 of the 12 recorded approaches were to yellowish-orange females (Fig. 1), which represents a significant difference (Chi-square goodness of fit to random-expected frequencies: $\chi^2_1=8.3$, $p<0.005$). Males therefore preferentially approached yellowish-orange females in spring (the small total number of approaches limits further statistical analysis of this sample). Males also preferred to approach yellowish-orange females in summer ($N=308$ approaches to yellowish-orange females vs $N=86$ to alba females; $\chi^2_1=125.1$, $p<0.001$). Moreover, the ratio of approach types varied between phenotypes (Chi-square heterogeneity: $\chi^2_1=11.0$, $p<0.001$), with relatively more "contact" approaches directed towards yellowish-orange females (Fig. 1). These data strongly support the notion that yellowish-orange females are more "attractive" to mate-locating male conspecifics.

Female mating biology

Table 1 provides a breakdown of statistics regarding the mating biology of sampled females, and Fig. 2 indicates the frequency distribution of spermatophores held by females, grouped by season and phenotype. Not one of the total 308 sampled females was virgin, and the maximum mating frequency was four (seen in two orange females sampled in summer). The distribution of spermatophores did not differ among yellowish-orange and alba phenotype females, either in spring (Chi-square heterogeneity test: $\chi^2_1=0.093$, $p=0.76$) or summer ($\chi^2_2=2.02$, $p=0.36$).

We identified the best fitting ordinal multinomial model of spermatophore frequency using a best-subsets approach that initially included the predictor variables phenotype (alba or yellowish-orange), season (spring or summer), wing wear, forewing length, and all first- and second-order interactions (we did not include third-order interactions because this resulted in computational difficulties). The model that minimized the AIC value was significant ($G_2=32.1$, $N=308$, $p<0.001$; AIC=546.5), and included season and wing-wear as predictors. Both variables were significant predictors in this model (season-Wald=5.5, $p<0.05$; wing-wear-Wald=20.9, $p<0.001$). Given that season was coded 0=spring and 1=summer, a positive semi-partial correlation for season ($r=0.14$) indicates that females

carried more spermatophores in summer (Table 1). A positive semipartial correlation for wing wear ($r=0.25$) also indicates that females accumulate spermatophores with age (Fig. 3). The lack of effects due to phenotype, or phenotype×wing-wear, suggests that the lifetime accumulation of spermatophores proceeds equivalently for alba and yellowish-orange females.

We then identified the best-fitting linear model of mean spermatophore weight (starting with all predictor variables as above). The best fitting solution in this case included the variables season and phenotype ($G_2=14.5$, $N=308$; $p<0.001$; AIC=−2,099.88). Whereas season was a significant predictor (Wald=14.9, $p<0.001$), the effect of phenotype was not significant (Wald=2.77, $p=0.10$). The semipartial correlation for season ($r=−0.21$) indicated that mean spermatophore size was larger in spring, and the value for phenotype ($r=−0.09$) indicated a very weak pattern towards larger sper-
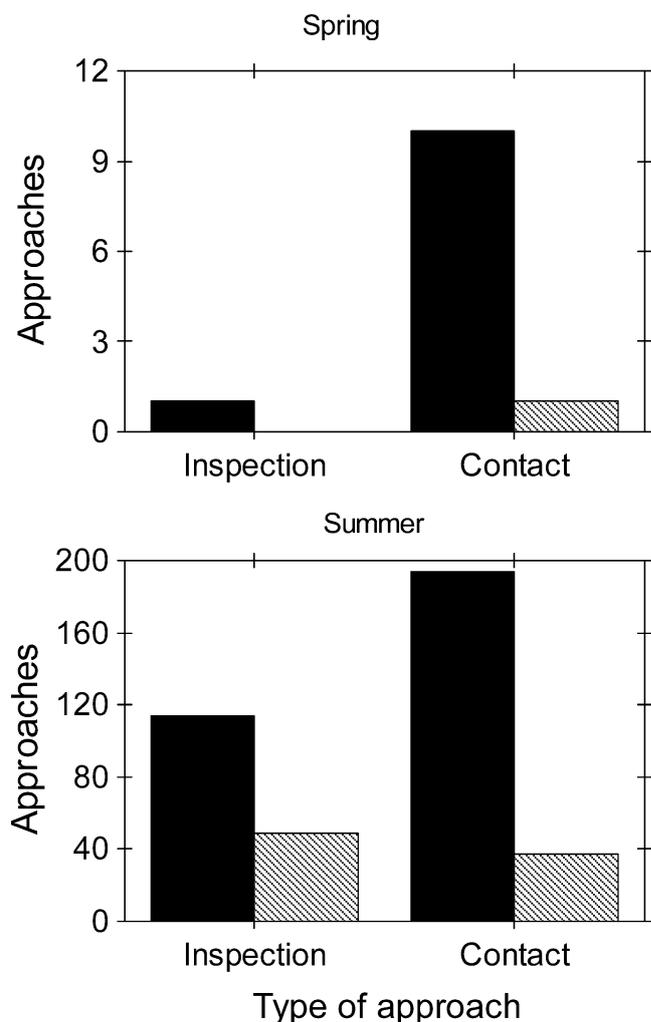

Fig. 1 Frequencies of approaches, grouped by approach type, for yellowish-orange phenotype (*solid columns*) and alba female models. This represents the result of 4 h (spring) and 8 h (summer) of total "observation" time for each phenotype. Approach types are explained in the text

Fig. 2 Distribution of spermatophores among yellowish-orange and alba females for each of the two seasons
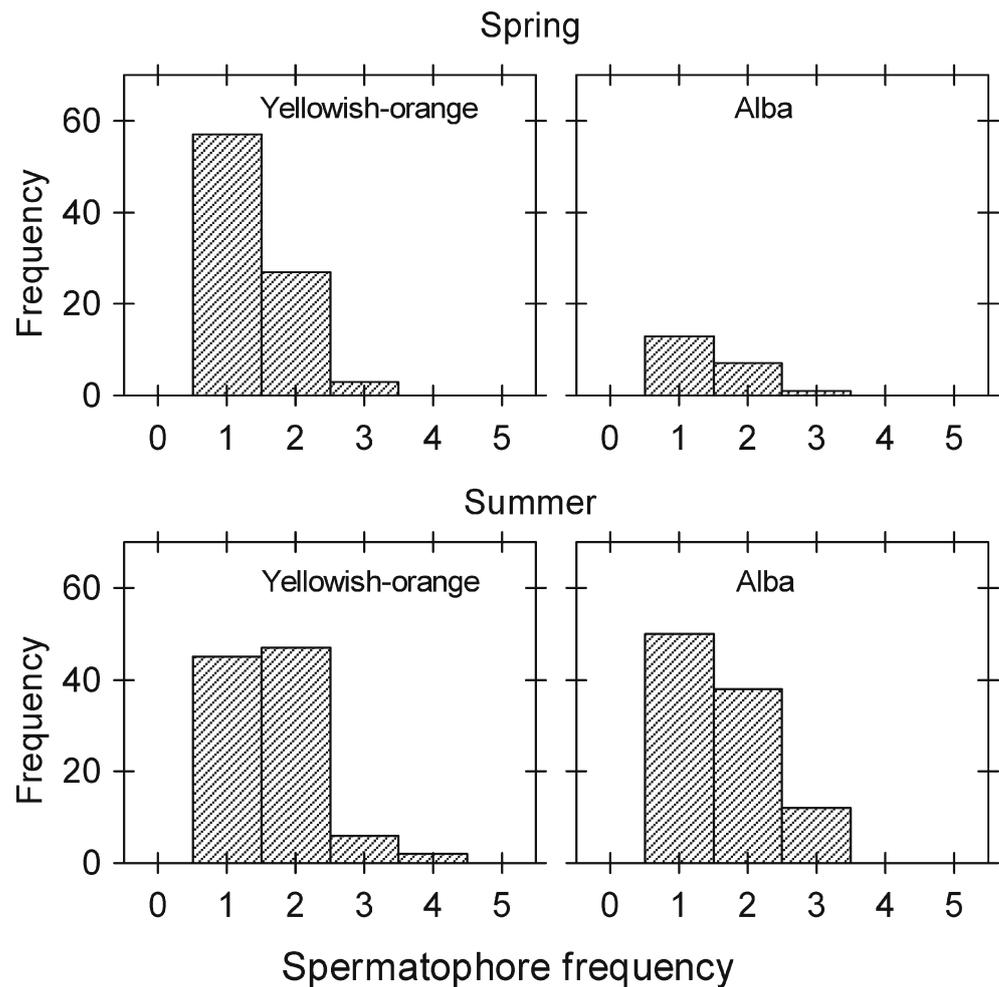
matophores in alba females (Table 1). Phenotype was a nonsignificant term in single-variable models conducted separately for spring ($G_1=0.80$, $p=0.37$) and summer ($G_1=2.89$, $p=0.089$), although alba's pattern for larger spermatophores is evident in the latter sample (see Table 1).
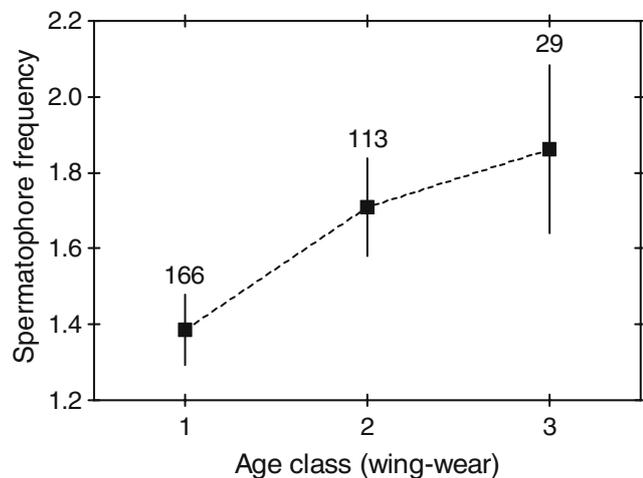
Fig. 3 Mean (±95% confidence interval) number of spermatophores held by differently aged females. Sample sizes are given above *upper error whiskers*. This pattern neither differs across phenotypes (alba/yellowish-orange) nor seasons (spring/summer; see "Results")

Discussion

Our investigation was designed to assess, first, whether male *C. eurytheme* possess a bias against the alba phenotype and, second, whether females of each phenotype differ in aspects of their reproductive biology, possibly as a consequence of such a bias. The data clearly demonstrate that mate-locating males are biased against alba females, which agrees with findings in other *Colias* species [most notably, the work of Graham et al. (1980) on montane *Colias alexandra* and *Colias scudderi*]. This further suggests that male mating biases are a general phenomenon among species that exhibit the alba polymorphism. Concurrently, however, we failed to find strong or statistically significant differences among female phenotypes in the distribution or frequency male-derived spermatophores, and there was, if anything, a weak pattern for alba females to carry larger spermatophores. Females did accumulate spermatophores with age (wing-wear), which is consistent with the findings of prior

investigations in *Colias* (e.g., Graham et al. 1980; Rutowski et al. 1981; Gilchrist and Rutowski 1986; Ellers and Boggs 2003), but this process appeared to proceed equivalently for females of each phenotype. Our findings therefore contrast with the clearly lower standing spermatophore frequencies previously reported for smaller samples of alba *C. alexandra* and *C. scudderi* (Graham et al. 1980), but are more consistent with those of Gilchrist and Rutowski (1986) regarding *C. eurytheme* (who report equivalent spermatophore frequencies among forms in all but the oldest wing-wear class). Thus, the present data may further highlight an interesting interspecific difference regarding alba reproductive biology. Our data also indicate that females of both phenotypes carried fewer, larger spermatophores during the cooler, low-population-density spring conditions. We discuss these findings in light of the known thermally and density-dependent complexities of alba reproductive biology and of the *Colias* mating system.

On face value, our data indicate a strong male mating bias that is not matched by strong differences in the frequency or mean weight of female-held spermatophores. The simplest candidate explanation is that the male bias has limited ultimate reproductive consequence, even under low-density spring conditions, due to the highly male-biased OSR of this population (which is supported by the fact that not one of our 308 sampled females was virgin). However, the extensive work of Watt and colleagues (Watt 1973; Graham et al. 1980; Nielsen and Watt 1998, 2000) on the alba polymorphism and Rutowski and colleagues on the *Colias* mating system (Rutowski et al. 1981, 1987; Rutowski 1985; Rutowski and Gilchrist 1986) suggests several ways in which this conclusion could be overly simplistic. First and foremost, the known resource advantage of alba females may see them as being less reliant on male-derived nutrients for reproduction, and to consequently erode spermatophores more slowly than their yellowish-orange counterparts (for which there may be some evidence, based on the weak tendency for larger spermatophores in alba females). If this is true, and if alba females receive average-sized spermatophores, then the existing model of *Colias* reproductive biology would predict a skew in the cost–benefit balance of mating (cf. Wiklund et al. 2001) for alba females in the direction of mating less frequently. This is a possible scenario for *C. alexandra* and *C. scudderi*, in which alba females do mate less than yellowish-orange females. On the other hand, if alba females are subject to greater latencies until mating, they may themselves be less choosy (Rutowski 1985) and tend to receive smaller spermatophores on average (which would then represent an "indirect" negative effect of the male mating bias). Under this scenario, alba female mating rates may approach those of yellowish-orange females, and depending on the average donated spermatophore size and rates of subsequent erosion, the mean "standing" spermatophore weight (as it was sampled here) could also be indistinguishable among forms. The male bias could therefore, somewhat paradoxically, contribute to gross equality in the mating dynamics of the two phenotypes, such as we have observed in this study.

Second, we sampled butterflies from each of two seasons that differed markedly in population density (as indicated by our model approach rates; Fig. 1), but individuals in each season also experienced different juvenile and adult thermal regimes (see "Materials and methods"). Temperature may be of consequence because both the juvenile physiological advantage and adult flight patterns of the alba phenotype are thermally dependent (Graham et al. 1980; Nielsen and Watt 1998). Again, the potential outcomes of temperature and/or density effects are likely to be complex and species-specific (Nielsen and Watt 2000), but the clearest prediction for *C. eurytheme* would seem to be for divergence between alba and yellowish-orange female mating patterns in spring. Lower springtime population densities should increase the alba female "lag time" for initial mating (Nielsen and Watt 2000), which would be further exacerbated if alba females require more thermally restrictive conditions for flight (Nielsen and Watt 1998). This should lead to a greater likelihood of sampling a virgin female alba in spring (which we did not find, although we did begin sampling after the start of the spring flight season). Second, the putatively greater resource allocation advantage of alba under cooler conditions (Graham et al. 1980) and reduced opportunity for flight are two reasons to suggest alba females should use their male-derived nutrients even more slowly than their yellowish-orange counterparts in spring (thus contributing to lower mating rates). Given these (and other) potentially mitigating seasonal factors, it is surprising that we failed to detect strong seasonal variation in mating dynamics between the two female phenotypes. Our data do, however, indicate interesting seasonal differences in the mating dynamics of all females, with springtime females seen to carry fewer, heavier spermatophores. This finding is consistent with them either receiving initially larger spermatophores or subsequently eroding them more slowly (perhaps because of lower activity under cooler conditions; Nielsen and Watt 1998).

The extensive work of Watt and colleagues on the alba phenotype suggests that the maintenance of this polymorphism is a very complex issue (see, e.g., the modeling approach of Nielsen and Watt 2000). Our present data address a single variable in this equation (the potentially negative effects of male mate choice), and thus bear only partial relevance to the broader issue of net selection on the different female phenotypes. A more interesting and potentially enlightening feature of our findings concerns the apparent difference in female mating biology between *C. eurytheme* and the other well studied exemplars of the alba polymorphism (i.e., *C.*

alexandra and *C. scudderi*). The male mating bias is apparently common to all of these species; yet, *C. eurytheme* alba do not exhibit the relatively lower spermatophore frequencies observed in the other two species. Owing to our relatively large sample size (N=309 vs N=143 and N=97 of the significant *C. alexandra* and *C. scudderi* effects, respectively; Graham et al. 1980), our present analysis is unlikely to have suffered from low statistical power for detecting such a difference. Hence, this intraspecific comparison at least underscores that female mating rates are influenced by more than just simply male mating biases. Understanding the extent of the difference among species may require full consideration of their thermal, nutritional, and demographic differences. A simple starting point for furthering this comparison and generating more specific hypotheses may be an investigation into average spermatophore weight in each of the two *C. scudderi* and *C. alexandra* female phenotypes.

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