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## HERITABILITY OF DEVELOPMENT TIME AND PROTANDRY IN THE PITCHER-PLANT MOSQUITO, *WYEOMYIA SMITHII*

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**Abstract.** Models of protandry (1) assume implicitly or explicitly that independent evolution of male and female preadult development times is possible and (2) assume or assert that protandry should increase in populations that are univoltine or at least composed of discrete, nonoverlapping generations. Herein we examine these assumptions in the pitcher-plant mosquito, *Wyeomyia smithii*. Heritability and additive genetic variance of development time are higher in female than in male *W. smithii*. Protandry is higher in lines selected for slow development than in lines selected for fast development or in unselected control lines. Protandry is therefore capable of evolving. Contrary to predictions based on sexual selection, southern populations with multiple, overlapping generations are just as protandrous as northern bi- and univoltine populations with discrete generations. Voltinism and developmental synchrony of the population do not appear to have been major selective factors in the evolution of protandry in *W. smithii*. We propose that protandry can be maintained by natural selection in multivoltine populations with overlapping generations as a consequence of sexually dimorphic fitness criteria. Selection should minimize development time in males but maximize growth rate in females. In *W. smithii*, females achieve higher growth rate than males but also harbor greater genetic variation for development time, indicating that selection has, indeed, minimized development time to a greater extent in males than in females. We conclude that if both natural and sexual selection are involved in the maintenance of protandry in populations of *W. smithii*, then their relative importance changes with the degree of generation overlap.

**Key words:** biogeography; evolution; heritability; mosquito; pitcher plants; protandry; sexual selection; voltinism; *Wyeomyia*.

### INTRODUCTION

Herein we assess the genetic potential for the evolution of protandry in the pitcher-plant mosquito, *Wyeomyia smithii* (Coq.), and consider the effect of voltinism and synchrony of seasonal development on the evolution of protandry. Protandry, the emergence of males before females into a seasonal breeding population, has long been observed in many insects and other arthropods. Protandry has also been reported for some mammal populations that have restricted breeding seasons, such as mountain hares (Iason 1989) and ground squirrels (Michener 1983). Both Darwin (1871) and Wallace (1889) discussed the early seasonal appearance of male insects as an example of sexual selection due to increased mating opportunity for early emerging males.

Sexual selection is selection on the relative ability of males to acquire and mate with females as contrasted with natural selection on male survivorship. The two forms of selection may act in concert if, for example, protandrous males outcompete later emerging males for

females and also live longer; the two forms of selection may be antagonistic if, in the process of emerging early, protandrous males are subject to higher predation.

More recently, mathematical models of protandry have been developed which describe protandry as a male mating strategy in response to sexual selection that maximizes the number of matings achieved by a male (Wiklund and Fagerström 1977, Bulmer 1983, Iwasa et al. 1983, Parker and Courtney 1983). Several models describe protandry as a mating strategy of both sexes where males are selected to maximize the number of matings and females are under selective pressure to minimize the prereproductive period, thereby decreasing mortality during this period (Scott 1977, Fagerström and Wiklund 1982, Zonneveld and Metz 1991). In addition, a few studies provide evidence that protandry is not an incidental side effect of some other process; rather, it stems from selection acting on both sexes (Singer 1982, Wiklund and Solbreck 1982, Forsberg and Wiklund 1988, Wiklund et al. 1991, Kleckner et al. 1995).

For protandry to evolve, all these models assume or assert that (1) the independent evolution of male and female preadult development time is possible, i.e., that protandry is a heritable trait and (2) populations are univoltine or at least comprised of discrete, nonoverlapping generations. Singer (1982) connected protan-

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dry with sexual size dimorphism and the synchrony of seasonal development. Singer assumed that selection for protandry is a form of sexual selection so that if there were overlap between generations, males could not be selected to emerge before females. Assuming that males and females realized the same preadult growth rates, selection for protandry (early emerging males) would then be causal to the female-biased size dimorphism. Thornhill and Alcock (1983) envisioned the reverse process, driven by natural selection. If large females achieve greatly increased fecundity but large males do not achieve disproportionate reproductive success, then there is direct (natural) selection on female-biased size dimorphism. Assuming a "developmental constraint" of a positive genetic correlation between size and development time, larger females would emerge later than smaller males and protandry would then be the result of natural selection for sexual size dimorphism. Nylin et al. (1993) sought to distinguish these alternatives by examining geographic variation in size, development time, and protandry in the butterfly *Pararge aegeria*. They found "strong protandry in strongly seasonal northern habitats where generations are few and discrete" and "weak protandry in the mildly seasonal habitat of Spain where generations overlap to a high degree." Further, they found that protandry measured as the absolute difference in male and female development time remained relatively constant over a range of temperatures and photoperiods that severely altered development time. They therefore concluded that protandry in *P. aegeria* was the result of sexual selection on protandry per se, and not the indirect result of natural selection on female-biased size dimorphism.

Herein, we pursue a similar line of enquiry as Nylin et al. (1993), but with the pitcher-plant mosquito, *Wyeomyia smithii* (Coq.). *Wyeomyia smithii* completes its preadult development only within the water-filled leaves of the purple pitcher plant, *Sarracenia purpurea* L. The range of the mosquito follows that of its host plant from the Gulf of Mexico to northern Manitoba and Labrador (Darsie and Ward 1981). Southern populations are multivoltine and, because of variable density-dependent development, experience overlapping generations throughout the growing season; northern bi- and univoltine populations experience reduced or little density-dependent larval development and generations are discrete (Lounibos and Bradshaw 1975, Bradshaw 1983, Bradshaw and Holzapfel 1983, 1986). Although there is precocious ovarian development in northern as compared to southern *W. smithii*, both northern and southern females achieve sexual maturation and are receptive to insemination at the same age (O'Meara and Lounibos 1981). Available biogeographic, morphological, and physiological evidence indicate that the direction of evolution in *W. smithii* has proceeded from south to north (Bradshaw and Lounibos 1977, Istock and Weisburg 1987, Bradshaw and Hol-

TABLE 1. Origin of populations.

Population†	Latitude (°N)	Altitude (m)	State or province
WI‡	30	10	North-central Florida
CR‡	31	30	Northwestern Florida
GS‡	34	20	Coastal North Carolina
EW	34	150	Piedmont North Carolina
DB	35	900	Alpine North Carolina
HK	35	900	Alpine North Carolina
PB	40	10	Coastal New Jersey
MM	40	10	Coastal New Jersey
FV‡	43	60	Eastern Massachusetts
HL	43	260	Southern Michigan
KC‡	46	110	North-central Maine
DL‡	49	370	Northwestern Ontario

† Population designation in previous studies (Bradshaw and Lounibos 1977, Bradshaw and Holzapfel 1989, 1990, Hard et al. 1993).

‡ Populations used in the selection experiments (Figs. 1–3A).

zapfel 1990). Consequently, if protandry is a heritable trait, then, as in Nylin et al. (1993) (1) protandry due to sexual selection should increase with latitude in this species as voltinism decreases and seasonal developmental synchrony increases and (2) the absolute difference in male and female development times should remain constant over a range of larval densities that alters preadult development time. By contrast, if protandry is maintained by natural selection on sexual size dimorphism, then protandry should remain consistent over the geographic range of *W. smithii* and vary with larval density. Below, we resolve these alternatives, first by establishing protandry as a heritable trait. We then show how development time and protandry vary among geographic populations and larval densities.

## MATERIALS AND METHODS

### *Selection and estimation of responses*

The methods for the collection of the populations, the establishment of the experimental lines, and the selection on development time are provided in Hard et al. (1993), who determined the heritability of development time in these same six populations, but without regard to sex. Approximately 2000 larval *W. smithii* were collected from the 1987–1988 overwintering generation at each of six localities in eastern North America from 30° to 49°N (Table 1). We transported the larvae to the laboratory and, to minimize residual field effects, reared populations to the F<sub>2</sub> generation of wild-caught mosquitoes before initiating selection on development time.

We imposed selection and determined direct and correlated responses in cohorts of *W. smithii* raised in the leaves of intact pitcher plants placed in controlled environment rooms designed to simulate physical conditions in nature. Light consisted of long-day photoperiods (L:D = 18:6 with a 0.5-h transitory twilight at both dawn and dusk) to promote continuous develop-

ment (nondiapause) in all populations and to avoid photoperiod–development time interaction. Temperature consisted of a smooth, sine-wave thermoperiod with a daily range of 13°–29°C and mean of 21°C; the phase of the thermoperiod lagged that of the photoperiod by 3 h. Relative humidity was maintained at a constant  $80 \pm 5\%$ . Food (whole, freeze-dried *Drosophila melanogaster* Meigen) was provided to each developing cohort over a 3-wk period to mimic the natural prey-capture cycle of host leaves (Bradshaw 1983).

For each of the six populations, we established three lines: selection for fast development (oviposition to adult eclosion), selection for slow development, and an unselected control. To synchronize development within and between all lines, the offspring of each selected generation were grown in petri dishes on short days (L:D = 8:16) at a constant 21°C to induce diapause. After the offspring of the selected generation were synchronized in diapause, that diapause was terminated abruptly by transferring the larvae to long days in the controlled-environment room. Developing larvae were reared to adulthood and their resulting offspring reared in leaves and again subjected to selection. Thus, selection was imposed only every other generation, but this technique permitted (1) simultaneous selection by truncation for a temporal character in diverging lines, (2) increased probability of random mating with respect to development time before selection was imposed, and (3) reduced linkage disequilibrium resulting from selection and assortative mating that may have occurred within selected lines. Average effective population size ( $1/N_e = 1/4N_\delta + 1/4N_\varphi$ ) varied from 51 to 97 and the cumulative inbreeding coefficients ranged from 3.4 to 8.4% over the selection process (Hard et al. 1993:Table 1).

Each selected line in each population and selected generation consisted of 400 larvae (20 larvae reared in each of 20 leaves). Since *W. smithii* spend 4–5 d as a pupa, we were able to collect, separate, and save each line's production of male and female pupae near the truncation time. This procedure gave us a 4–5 d window to assess the selection differential generated by imposing the truncation point on a given day, thereby increasing our ability to equalize the intensity of selection on each sex within each selected line. We were then also able to determine the intensity of selection, the heritability, and the additive genetic variance of development time separately for each sex. The sixth generation of selection was completed in the  $F_{12}$  laboratory generation, lines were synchronized by inducing diapause in the  $F_{13}$  laboratory generation, and the heritability and additive genetic variance of development time were determined in the  $F_{14}$  generation (Hard et al. 1993).

#### *Heritability and additive genetic variance*

The realized heritability ( $h^2$ ) of development time was calculated from the ratio of total selection response

( $R$ ) to total selection differential ( $S$ ) after six generations of divergent selection (Hill 1972):  $h^2_{DT} = R/S$ . The variance of the heritability was calculated by:  $\sigma^2(h^2_{DT}) = \sigma^2(R)/S^2$ , where  $\sigma^2(R)$ , the variance in response, was estimated as in Hard et al. (1993). The intensity of selection was calculated as  $i = S/\sigma_z$  and the additive genetic variance as  $V_A = h^2_{DT} \times \sigma_z^2$  for each population and sex, where  $\sigma_z$  is the phenotypic standard deviation of development time for each sex in each population before selection (i.e., in the laboratory  $F_2$ ).

Heritabilities were scored as significantly greater than zero if  $h^2_{DT} - 2\sigma(h^2_{DT}) > 0$ . The species-wide difference in male and female heritabilities was tested by a  $t$  test for paired comparisons ( $h^2_{\varphi DT} - h^2_{\delta DT}$ ) using each population as an independent replicate (i.e., 5 df). Species-wide differences between male and female intensities of selection and additive genetic variances were tested using this same procedure. Differences between heritabilities of males and females within populations were then considered significant if  $h^2_{\varphi DT} - 2\sigma(h^2_{\varphi DT}) > h^2_{\delta DT}$  and vice versa.

#### *Direct response of development time to selection*

The  $F_{15}$  generation was again synchronized in diapause. The direct response of development time and the indirect response of protandry were determined at low density in the  $F_{16}$ ; then, using the  $F_{16}$  as parents, the indirect response of development time and protandry were determined at high, limiting density in the  $F_{17}$  as in Bradshaw and Holzapfel (1996). Pupae from five replicate cohorts were pooled so that each selected line in each population was represented by a single adult cage (18 total cages).

Because we sexed and pooled pupae prior to adult emergence, development time was calculated as log (days from oviposition to pupation). We treated population as a random effect and used a model II ANOVA to test for significant effects of selected lines, larval density, sex, and their two- and three-way interactions, with replicate cohort means (from individual leaves) for each sex as independent observations within populations.  $F$  values were calculated as the treatment mean square divided by the mean square for treatment by population interaction. A posteriori testing of treatment means used Ryan's  $Q$  pairwise comparisons to control for experimentwise error rates (Day and Quinn 1989).

#### *Protandry*

We calculated protandry for each cohort (leaf) as mean log(female development time) – mean log(male development time). We treated population as a random effect and used a model II design to test, as above, for significant effects of selected lines, density, and line by density interaction. A posteriori testing of line or population means used Ryan's  $Q$  pairwise comparisons

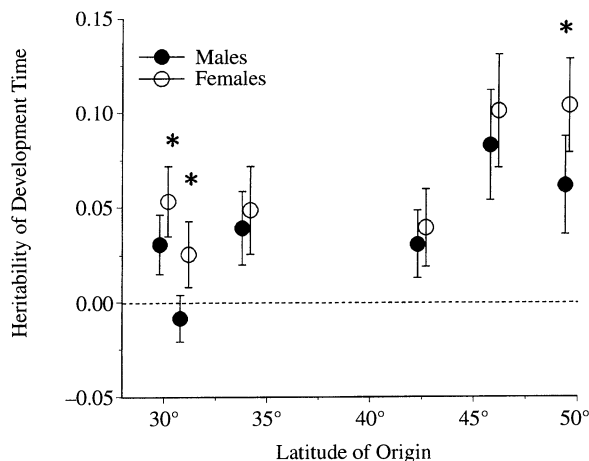


FIG. 1. Heritability ( $\pm 2$  SE) of development time in males and females. Asterisks (\*) indicate individual populations in which male and female heritabilities differ by  $> 2$  SE of either sex.

to control for experimentwise error rates (Day and Quinn 1989).

Variation in protandry among populations was assessed by nested ANOVA using the GLM procedure (SAS 1985). The treatment mean square was obtained as the mean square among populations within lines and densities; the error mean square was obtained as the mean square among leaves within populations. Although there were exactly five cohorts (leaves) per density  $\times$  line  $\times$  population, one leaf failed to produce female pupae, leaving 30 df for the treatment mean square (2 densities  $\times$  3 lines  $\times$  [6 - 1] populations within densities and lines) and 143 df [(2 densities  $\times$  3 lines  $\times$  6 populations  $\times$  [5 - 1] leaves) - 1 = 143] for the error mean square.

The above analysis of protandry among populations included lines selected for development time. To make an independent assessment of geographical variation in protandry among unselected populations, we examined the 12 populations considered by Bradshaw and Holzapfel (1989, 1990), which included the same six populations in the present study but represented independent collections made 9 yr earlier. In this case, each population had experienced 5-6 laboratory generations without any intentional selection on development time or protandry. The larvae were reared under the same conditions of temperature, photoperiod, and humidity as above but were pooled as pupae and sexed at adult emergence from the pupal exuviae. Consequently, development time was measured as days from oviposition to adult emergence. This procedure produced one estimate of protandry for each of 12 populations reared at each of four larval densities with pooled cohorts of 100-240 larvae per population times density. We calculated protandry two ways.

First, we calculated protandry for each population as mean  $\log(\text{female development time}) - \text{mean}$

$\log(\text{male development time})$ . Two populations originated from alpine localities in the southern Appalachians. To correct for altitude in these populations, we used the altitude : latitude correction factor of 142 m: 1°N based on photoperiodic response in *W. smithii* (Bradshaw 1976, Bradshaw and Lounibos 1977).

Second, to examine absolute difference in development time of males and females across a variable environment (larval density) we calculated protandry as (mean development time females - mean development time males), which we then analyzed nonparametrically by an extension of the Kruskal-Wallis test (Zar 1984: 219-222).

## RESULTS

Heritability of development time (Fig. 1) estimated from the divergence of the fast and slow lines was higher in females than in males ( $t$  test for paired comparisons:  $t = 4.13$ ;  $df = 5$ ;  $P < 0.01$ ) despite the lower intensity of selection on females than males (Table 2). Within individual populations, the heritability of development time differed (nonoverlap of either mean with 2 SE of the other mean) between males and females in the two extreme southern populations and in the extreme northern population. The estimated additive genetic variance for development time was higher in females than in males (Table 2).

After selection on development time at low density, mean development time in the  $F_{16}$  and  $F_{17}$  generations (Fig. 2) showed significant effects of selected line ( $F_{2,10} = 6.84$ ;  $P < 0.05$ ), larval density ( $F_{1,5} = 61.93$ ;  $P < 0.001$ ), sex ( $F_{1,5} = 166.35$ ;  $P < 0.001$ ), line by sex interaction ( $F_{2,10} = 8.54$ ;  $P < 0.01$ ), and density by sex interaction ( $F_{1,5} = 12.46$ ;  $P < 0.05$ ), but not line by density interaction ( $F_{2,10} = 0.36$ ;  $P > 0.05$ ) or the three-way interaction ( $F_{2,10} < 0.01$ ;  $P > 0.05$ ). Ryan's  $Q$  pairwise comparisons showed (Fig. 2, inset) that male development time was faster in the lines selected for fast development than in the slow or control lines but did not differ between the latter two lines; female development time was faster in the lines selected for fast development than in the control lines in which devel-

TABLE 2. Intensity of selection ( $i$ ) and additive genetic variance ( $V_A$ ) of development time =  $\log(\text{days oviposition to adult eclosion})$  by sex in each population (Pop.; from Table 1) of *W. smithii*.

Pop.	$i$		$V_A \times 10^{-5}$	
	$\delta$	$\text{♀}$	$\delta$	$\text{♀}$
WI	16.83	14.27	6.60	15.69
CR	16.95	12.11	0.00†	10.23
GS	14.07	11.84	15.00	26.33
FV	16.06	13.95	9.12	15.54
KC	11.67	11.32	65.84	85.38
DL	9.55	9.90	35.54	55.47
$t\ddagger$	2.63; $P < 0.05$		5.53; $P < 0.01$	

† Actually estimated as -1.87.

‡  $t$  test for paired comparisons with 5 df.

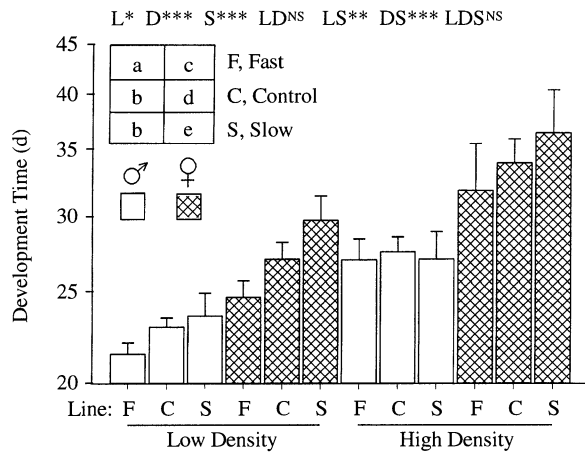


FIG. 2. Effect of selection for fast or slow development at low larval density on development time at low and high larval density. The bars plot mean + 2 SE of development time (days oviposition to pupation) of males (open bars) and females (shaded bars) from the fast (F), slow (S), or unselected control (C) lines. The top of the figure shows the effects on development time of selected line (L), larval density (D), sex (S), and their two-way (LD, LS, DS) and three-way (LDS) interactions: <sup>NS</sup>  $P > 0.05$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ . Insert: Ryan's  $Q$  pairwise comparisons among lines and sexes; means corresponding to cells with the same letter do not differ ( $P > 0.05$ ).

oment was faster than in the lines selected for slow development. These results show (1) that males develop faster than females, i.e., *W. smithii* is protandrous, (2) that the relative development time of males and females is affected by larval density, implying that protandry is density dependent, and (3) that direct selection on development time has a direct effect on development time that is independent of density but not sex, implying that protandry is heritable.

After selection on development time at low density, protandry (Fig. 3A) showed significant effects of larval

density ( $F_{1,5} = 11.91$ ;  $P < 0.05$ ) and selected line ( $F_{2,10} = 8.84$ ;  $P < 0.01$ ) but not line by density interaction ( $F_{2,10} = 0.14$ ;  $P > 0.05$ ). Following ANOVA, Ryan's  $Q$  pairwise comparisons on species-wide means revealed that protandry was greater in the slow than control ( $P < 0.05$ ) or fast lines ( $P < 0.01$ ), which did not differ from each other ( $P > 0.05$ ). These results show that protandry was altered by selection on development time.

Among the six populations, there was significant variation in protandry among populations within lines and densities ( $F_{30,143} = 3.18$ ;  $P < 0.001$ ). Ryan's  $Q$  pairwise comparisons revealed that protandry was shorter ( $P < 0.05$ ) in the population from Maine (46°N) than the three southern populations (30°–35°N) (Fig. 3B); none of the other populations differed in protandry ( $P > 0.05$ ) within or between regions. Among the 12 unselected populations used earlier (Bradshaw and Holzapfel 1989, 1990), protandry was positively correlated with density ( $t = 4.35$ ;  $P < 0.001$ ) but not latitude of origin ( $t = -0.78$ ;  $P > 0.05$ ). A plot of the residuals from regression of protandry on density showed that the nonsignificant trend was toward shorter protandry in the more northern populations (Fig. 4). These results show that protandry does not increase with latitude in *W. smithii*.

To test for absolute differences in male and female development time, we performed a two-way Kruskal-Wallis test on the ranked differences in mean male and female development time. As in Bradshaw and Holzapfel (1989) we divided the 12 populations into six geographic zones with two independent localities at each of the four densities (10, 20, 40, and 60 larvae per leaf). The Kruskal-Wallis test revealed a significant effect of density on protandry ( $H = 13.88$ ;  $df = 3$ ;  $P < 0.01$ ) but not geographic zone ( $H = 5.25$ ;  $df = 5$ ;  $P > 0.05$ ) or density by zone interaction ( $H = 5.88$ ;  $df = 15$ ;  $P > 0.05$ ). These results show that the absolute

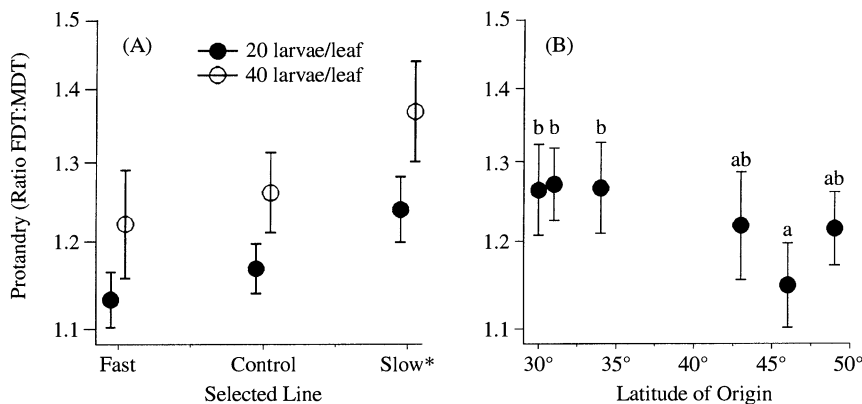


FIG. 3. Protandry, calculated as the ratio of female : male development time (FDT:MDT; mean ± 2 SE) after selection for fast and slow development with an unselected control. (A) Protandry at low (20 larvae/leaf) and high (40 larvae/leaf) larval densities. The asterisk (\*) indicates a line different ( $P < 0.05$ ) from the others. (B) Latitudinal variation in protandry ranging from southern, multivoltine populations with overlapping generations to northern, univoltine populations with synchronous generations. Means accompanied by the same letter do not differ ( $P > 0.05$ ) from each other.

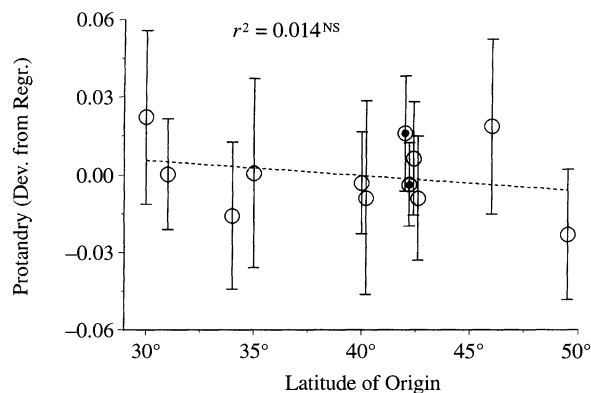


FIG. 4. Protandry [ $\log(\text{development time of females}) - \log(\text{development time of males})$ ; mean  $\pm$  2 SE] in unselected lines from 12 localities. Protandry is plotted as mean deviation from regression of protandry on larval density. The solid dots within the two open circles identify the alpine populations from North Carolina.

difference in male and female development time varies over a range of larval densities that alters preadult development time.

#### DISCUSSION

In *Wyeomyia smithii*, there is a female-biased heritability for development time (Fig. 1). This bias leads to greater direct response to selection on development time in females than in males (Fig. 2) and, consequently, an increase in protandry in lines selected for slow development (Fig. 3A). Protandry is therefore a heritable trait, capable of responding to selection in *W. smithii*. Nonetheless, contrary to predictions from sexual selection theory (Singer 1982, Nylin et al. 1993), protandry persists in southern multivoltine populations with overlapping generations and does not increase with latitude as populations tend towards synchronous generations and univoltinism (Figs. 3B and 4). We therefore find no evidence that sexual selection has played a role in the evolution of protandry as *W. smithii* has expanded its range northward in North America.

Nylin et al. (1993) argued that, if protandry were maintained by sexual rather than natural selection, the absolute difference in male and female emergence should remain constant across environments that alter preadult development time. This argument is based on the assumption that all females are of equal value and that male fitness is measured by the number of mates acquired. In mosquitoes, this assumption does not necessarily hold in populations with synchronous development and variable larval density. As larval densities increase, preadult development time also increases but adult size, and consequently, female lifetime fecundity decreases (Moeur and Istock 1980, Bradshaw and Holzapfel 1990, 1992). There is then an increased premium on protandry so that the males can mate with the earlier emerging, more fecund females (Kleckner et al. 1995). In this situation, density-dependent protandry becomes

a norm of reaction whose shape may be molded by sexual selection. This argument does not, however, obviate the observation that in *W. smithii* neither the degree of protandry nor the reaction norm of protandry over variable larval density reflects voltinism or degree of seasonal synchrony, regardless of whether protandry is measured as the absolute difference in mean development time or as the log of the ratio of development times.

Singer's (1982) and Nylin et al.'s (1993) predictions based on sexual selection as well as Thornhill and Alcock's (1983) "developmental constraints" hypothesis based on natural selection all assume that the evolution of development time is constrained by a genetic trade-off with adult size. In *W. smithii*, direct selection on development time yields the direct response in faster or slower development (Fig. 2; Hard et al. 1993) but no correlated response in pupal mass or lifetime female reproductive success and the nonsignificant trend is toward higher mass and greater fecundity in lines selected for fast than slow development (Bradshaw and Holzapfel 1996). Consequently, there is no demonstrable trade-off or coevolution between development time and adult size or fecundity in *W. smithii*. The effects of selection on sex-dimorphic development time and adult size must be considered independently of each other.

We propose that protandry can be maintained by natural selection when each sex realizes fitness by independent criteria. Preadult development time is the primary determinant of variable generation time in *W. smithii* (Moeur and Istock 1980). In females, size-dependent fecundity is the primary determinant of lifetime offspring produced (Moeur and Istock 1980, Bradshaw and Holzapfel 1992, Bradshaw et al. 1993). In males, being small carries little cost to lifetime offspring sired (Benjamin and Bradshaw 1994). Hence, natural selection should minimize development time (and therefore generation time) in both sexes but maximize size (and therefore fecundity) in females, resulting in stronger selection on minimal development time in males and higher growth rate in females. Female *W. smithii* are larger than males and achieve higher growth rates (Bradshaw and Holzapfel 1996) but also harbor greater genetic variation for development time than males (Fig. 1, Table 2), indicating that selection has, indeed, operated to minimize development time more strongly in males than in females.

We do agree with Singer (1982) that sexual selection (maximizing the number of matings) on protandry should occur only when generations are discrete, because, if there are overlapping generations, males do not lose opportunity to mate by emerging later in the season. We cannot exclude the possibility that this form of sexual selection contributes to the maintenance of protandry in northern populations of *W. smithii*. In southern populations, we have had to invoke purely demographic arguments (natural selection) to explain

the maintenance of protandry with multiple, overlapping generations. We therefore conclude that if both natural and sexual selection are involved in the maintenance of protandry in populations of *W. smithii*, their relative importance changes with the degree of generation overlap.

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