PROTANDRY: THE RELATIONSHIP BETWEEN EMERGENCE TIME AND MALE FITNESS IN THE PITCHER-PLANT MOSQUITO

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Abstract. Protandry is the emergence or arrival into a seasonal population of males before females and is widespread among both plants and animals. Conceptually, protandry should be subject to stabilizing selection because early emerging males risk death before mating and late-emerging males miss opportunities to mate. However, for any given male, the optimal emergence time may depend upon the mean and distribution of emergence of other males in the population. Using lifetime offspring sired as our criterion of reproductive success, we found that, in laboratory populations of the pitcher-plant mosquito, *Wyeomyia smithii*, there is, indeed, stabilizing selection acting on the optimal time for male emergence, and that the specific optimum does depend upon emergence time of other males in the population. In addition, male cohorts achieved greater reproductive success when their emergence was spread over several days rather than occurring in a single day. However, reproductive success did not differ between normal and superdispersed emergence distributions, suggesting that, under more variable, natural conditions, the specific pattern of male emergence may be far less important than variation in emergence time, per se.

Key words: evolutionary stable strategy (ESS); invasions; life-history evolution; male fitness; mosquitoes, emergence time and male fitness; reproductive success; seasonality; sexual selection; Wyeomyia smithii.

INTRODUCTION

Protandry, the seasonal emergence of males before females, was originally conceived by Darwin (1871) as a form of sexual selection that maximizes mating opportunities for males while minimizing the pre-reproductive period for females (Wiklund and Fagerström 1977, Fagerström and Wiklund 1982, Bulmer 1983, Iwasa et al. 1983, Parker and Courtney 1983, Zonneveld and Metz 1991, Taylor et al. 1998).

Both theoretical models and empirical studies state or assume implicitly that there is stabilizing selection on male emergence time because late-emerging males miss opportunities to sire offspring and males that emerge too early risk death before they have the opportunity to mate. However, for any given male, the emergence time that maximizes his contribution to the next generation may depend on the timing and pattern of emergence of other males in the population (Iwasa et al. 1983, Parker and Courtney 1983, Thornhill and Alcock 1983, Zonneveld 1996). Because evaluating the lifetime number of offspring sired by males is time consuming or difficult, empirical studies use correlates of male reproductive success such as access to females or opportunities to mate (Botterweg 1982, Michener 1983, Parker and Courtney 1983, Elgar and Pierce 1988, Hastings 1989, Wang et al. 1990, Zonneveld 1992, Wiklund et al. 1996, Carvalho et al. 1998), mating success (Wiklund and Fagerström 1977, Iwasa et al. 1983, Schneider 1997, Castillo and Núñez-Farfán 1999), or potential offspring sired (Kleckner et al. 1995, Sawada et al. 1997, Carvalho et al. 1998). To our knowledge, no studies have measured actual lifetime reproductive success of males as a function of male emergence time. Here we use lifetime offspring sired by the pitcher-plant mosquito, *Wyeomyia smithii*, to test (1) whether there is an optimal time for male emergence relative to females, (2) whether this optimum depends upon the emergence times of other males, and (3) whether the pattern of variation of male emergence, independently of mean protandry, affects male reproductive success.

MATERIALS AND METHODS

Collection, maintenance, and common experimental conditions

Overwintering larvae were collected in Maine (USA) and on the Florida Panhandle (USA) (localities KC and WI, respectively, from the authors' previous publications). Populations were run through 6–8 generations in the laboratory to minimize field effects before the onset of experiments. Stock colonies were maintained in diapause as in Bradshaw (1986). When reared at low density and high larval resources in the laboratory, these two populations are fully interfertile, and hybrid fecundity and fertility do not exhibit any non-additive genetic differences between parent populations (Armbruster et al. 1997). Neither population of *Wyeomyia smithii* requires or will take a blood meal for at least the first ovarian cycle (O'Meara et al. 1981), obviating

the need for a vertebrate host for rearing, maintenance, or experiments.

Unless otherwise stated, all experiments were run in a controlled-environment room mimicking mid summer conditions common to both populations: unambiguous long-day photoperiod (L:D = 18:6) including two 0.5-h twilights, a daily sine-wave thermoperiod (12–28°C, mean = 21°C) which lagged the photoperiod by three hours, and 80 ± 5% relative humidity (Bradshaw 1986). Larvae of both populations were mass reared at low density and with high food levels provided ad libitum in the controlled-environment room and cleaned, fed, and checked for pupae three times per week (Monday, Wednesday, and Friday [M, W, F]). Pupae of each population were segregated by sex, maintained in separate dishes, and checked daily for adult eclosion. In each experiment, a cohort of 20 "invader" (Maine) males was introduced into a cage containing a cohort of 20 "native" (Florida) males and 20 native females. Eggs were collected from their common cage throughout the lifetime of all adults, and the resulting larvae reared to the third instar. The Florida larvae have four long anal papillae (used in salt and water balance), the Maine larvae have two short ventral papillae, and their F_1 hybrids have two long ventral and two short dorsal papillae that are morphologically indistinguishable from geographically intermediate populations (Bradshaw and Lounibos 1977). The larvae were scored as sired by a native male (four long anal papillae) or an invader male (two long and two short papillae). The relative success of the invader males over the native males was then measured as invader advantage:

$$IA = \frac{\text{Hybrid offspring} - \text{Native offspring}}{\text{Total offspring}}. \quad (1)$$

Each adult cage consisted of a screen cylinder (200 mm high, 150-mm diameter) lined on the bottom with filter paper kept moist with distilled water. Adults had constant access to raisins as a carbohydrate source and a 70-mL glass jar containing an ethanolic extract of *Sarracenia purpurea* leaves plus three leaf fragments, each ~1 cm², to stimulate oviposition. Dead adults were removed daily; eggs were collected three times a week (MWF) and the resulting larvae reared on short days (L:D = 8:16) at a constant 21°C.

Data analyses used Microsoft Excel 97 (Microsoft, Inc., Redmond, Washington) for regressions and one-way ANOVA and the SAS GLM procedure (SAS 1985) for two-way ANOVA. When significant treatment effects were indicated by ANOVA, differences among three or more individual means were evaluated with Ryan's Q multiple-comparison tests (Day and Quinn 1989).

Experiment 1: Varying mean with no variation in emergence times

The object of this experiment was to determine the effect on invader advantage when the degree of pro-

tandry varied but the variation in male and female emergence times was held constant at zero. The basic approach was to mass rear large numbers of both native and invader populations and to release adult cohorts of invader males, native males, and native females into a common adult cage; all individuals within each of the three cohorts had eclosed within 48 h of each other, and native- or invader-male cohorts were each placed in the adult cage on a single day but at varying combinations of times with respect to the native-female cohorts. This regimen enabled us to vary invader and native protandry independently, with zero variation in emergence time within any male or female cohort.

We used a full-factorial design with native protandries of 9, 6, 3, 0, −3, and −6 d crossed times invader protandries of 12, 9, 6, 3, 0, −3, and −6 d with 2–4 replicates each, except: (native, invader) no replicates of (0, 12), (−3, 12), (−6, 12), and (6, −6); one replicate only of (3, −6), (−3, −6), and (−6, −6). The total number of replicates was then 95. Note that negative protandry values represent male emergence after female emergence.

To evaluate native- and invader-male longevity, a subpopulation of northern males was reared to the fourth instar and then transferred to dishes containing 10^{-6} g/L Giemsa stain. Chronic exposure to this concentration of stain does not affect individual fitness but marks individuals for life (Kleckner and Bradshaw 1991). We used this procedure on a subset of 32 replicates wherein we collected dead adults daily, sexed them under a microscope, squashed individual males, and scored them as pale natives or blue invaders. We then calculated mean adult-male life span as days from day of introduction into the cage until mean male death.

Experiment 2: Constant mean and varying distribution of male emergence

The object of this experiment was to determine the effect on invader advantage of varying the distribution of native and invader emergence times while holding constant the distribution of female emergence times and holding constant the degree of native and invader protandry. The basic approach was to release adults within each cohort over varying periods of time to simulate three different emergence distributions: (1) "point": all 20 adults released on the same day; (2) "normal": 2, 4, 8, 4, and 2 adults released at five successive 3-d intervals; and (3) "superdispersed": 4 adults released at five successive 3-d intervals.

All females "emerged" with a normal distribution. Native and invader males emerged with point, normal, and superdispersed distributions in a full-factorial design (9 treatments) with 4 replicates each (36 total replicates). Based on the results of Experiment 1, we sought to offset an intrinsic invader-male advantage by fixing invader-male emergence on the median day of female emergence (protandry = 0 d) and median native-male emergence 3 d earlier (protandry = 3 d).
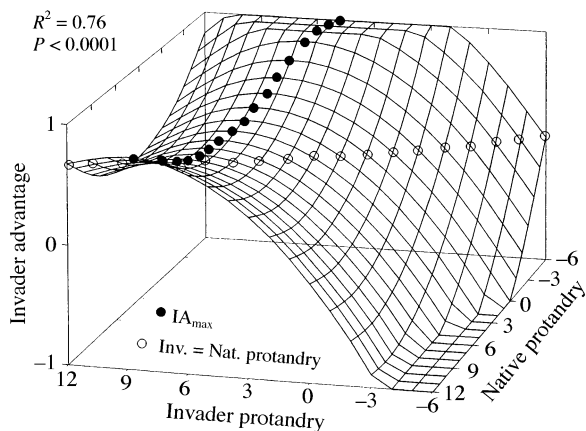
FIG. 1. Multiple-regression response surface showing invader-male advantage over native males with varying protandry of both native and invader males. Solid circles plot optimal invader protandry (IA_{max}) for each level of native protandry. Open circles plot invader advantage when invader protandry equals native protandry. Regression coefficients and their significance are in Table 1.

Larvae and adults were otherwise treated as in Experiment 1.

RESULTS

Experiment 1: Varying mean with no variation in emergence times

Invader advantage (Eq. 1) reflected both linear and quadratic effects of invader protandry and depended upon both native- and invader-male protandry and their interaction (Fig. 1). When native protandry was −6 d, invader advantage was maximal for an invader protandry of 4.8 d; as native protandry increased to 12 d, the invader protandry resulting in the highest invader advantage increased monotonically to 9.2 d (Fig. 1, solid circles). These results show that for each native protandry, there was an optimal invader protandry but that the position of this optimum relative to native-female emergence depended upon the degree of native protandry.

The response surface in Fig. 1 also shows that with equal native and invader protandry (open circles), the invader advantage was always positive. The mean adult life span of invader males (20.6 ± 0.6 d, $\bar{X} \pm 1$ SE) did not differ ($F_{1,62} = 0.051$, $P = 0.823$) from mean native-male life span (20.2 ± 1.2 d) and the residual
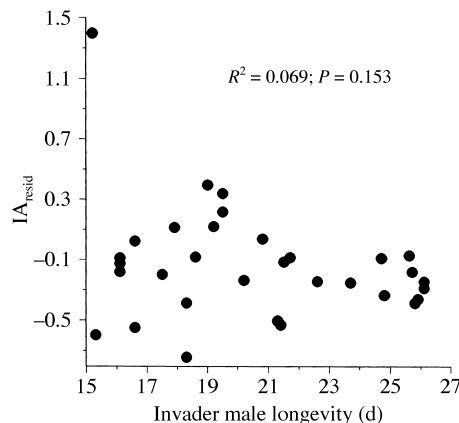

FIG. 2. Independence of invader advantage (IA) from mean invader-male longevity after the effects of native and invader protandry have been taken into account. IA_{resid} are the residuals from the regression of IA on native and invader protandry, their quadratics, and their interaction shown in Fig. 1.

invader advantage from regression in Fig. 1 was not significantly correlated with invader life span (Fig. 2). These results indicate that invader advantage did not depend upon male longevity and that Maine males possessed an intrinsic mating advantage over Florida males for Florida females, independently of adult-male longevity.

Experiment 2: Constant mean and varying distribution of male emergence

Invader advantage increased with variation in male emergence time (Fig. 3; two-way ANOVA: $F_{2,27} = 10.60$, $P < 0.001$) but was not affected by the native-male emergence distribution ($F_{2,27} = 1.68$, $P = 0.206$) or their interaction ($F_{4,27} = 0.110$, $P = 0.997$). Invader advantage was lower for the point distribution than either the normal or superdispersed distribution while the latter two did not differ from each other (Ryan's Q). These results show that variation in male emergence time at a fixed degree of protandry can enhance lifetime male reproductive success.

DISCUSSION

Regardless of native protandry, there is an optimal invader protandry (Fig. 1), confirming that protandry is indeed subject to stabilizing selection. The optimal protandry of invader males is dependent upon both

TABLE 1. Results of multiple regression of invader advantage (Eq. 1) on invader and native protandries, their quadratics, and their interaction, based on 95 total sample populations.

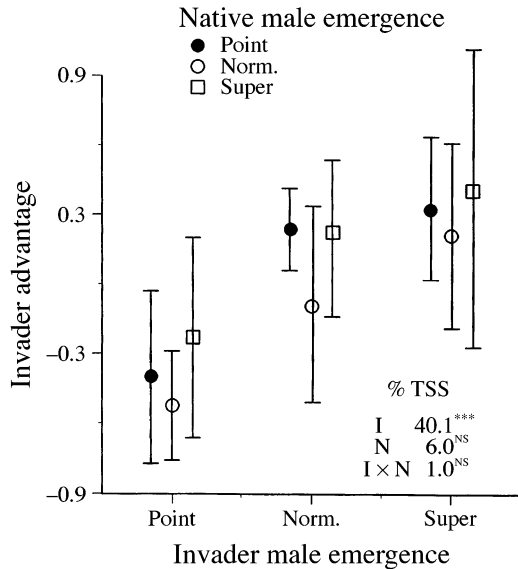| Source of variation	Coefficient	1 SE	t	P
Intercept	0.2285	0.0666	3.39	0.0010
Invader protandry	0.1393	0.0117	11.92	<0.0001
Native protandry	−0.1103	0.0105	10.49	<0.0001
(Invader protandry)²	−0.01112	0.00156	7.13	<0.0001
(Native protandry)²	0.006433	0.001684	3.82	<0.0003
Invader × Native protandry	0.005429	0.001601	3.39	0.0010

Fig. 3. Invader advantage when native protandry and invader protandry are fixed at 3 and 0 d before female emergence, respectively, but the distribution of both native and invader male emergence is varied. Point = all males released on a single day; Norm. = males released over five time intervals, approximating a normal distribution; Super = superdispersed distribution where one-fifth of the males were released over each of five time intervals. Also shown is the percentage reduction in total sum of squares (%TSS) in the ANOVA of invader advantage (and level of significance) with treatments (a) invader-male emergence distribution (I), (b) native-male emergence distribution (N), and (c) their interaction (I × N). Error bars represent ± 2 SE.

***$P < 0.001$; NS, not significant ($P > 0.05$).

their own protandry and the degree of native protandry (Fig. 1), thereby supporting theoretical models (Iwasa et al. 1983, Parker and Courtney 1983, Thornhill and Alcock 1983, Zonneveld 1996) indicating that fitness of an individual male is dependent not only on the timing of his emergence relative to females, but also on the timing of the emergence of other males in the population.

Models for the optimal emergence times of the sexes also predict (Wiklund and Fagerström 1977) that protandry should increase with male life expectancy. In *Wyeomyia smithii* we do not find a significant effect of invader-male longevity on invader advantage when both native and invader protandry have been taken into account (Fig. 2). In laboratory populations of *W. smithii* from Massachusetts reared under the same conditions as in this study, adult males that live longer encounter and mate with more females and, consequently, sire more lifetime offspring than shorter-lived males (Benjamin and Bradshaw 1993). However, when males are forced to fly in larger cages in search of females, longevity has a decreasing effect on lifetime offspring sired (Benjamin and Bradshaw 1994). Male longevity also decreases with increasing reproductive effort (offspring sired per female encountered), but lifetime off-

spring sired still increases with increasing reproductive effort (Benjamin and Bradshaw 1994). In the present case, any advantage of invader-male longevity may have been nullified by the flying of protandrous males in the frustrated search for females prior to female emergence, followed by a large reproductive effort by mating males once females were encountered.

In natural populations, male and female emergences are not restricted to points in time with zero variation. Rather, for both sexes, there is a temporal distribution in emergence and, for a given distribution of female emergence, there should be an optimal distribution of male emergence (Bulmer 1983, Iwasa et al. 1983, Parker and Courtney 1983, Thornhill and Alcock 1983, Sawada et al. 1997). In our experiments, we fixed the native-female emergence distribution as a normal distribution and determined whether invader-male emergence distributions with the same (normal), lesser (point), or greater (superdispersed) variation would achieve greater invader advantage. We found that invader advantage increased with variation of invader-male emergence, regardless of the native-male emergence distribution (Fig. 3). However, the normal and superdispersed invader-male emergence distributions did not differ from each other while both resulted in a higher invader advantage than the point (all adults released on same day) invader-male distribution. We obtained our results in a highly controlled environment with rigidly fixed mean invader and native protandries, and with carefully stylized emergence distributions. That we still could not resolve differences in fitness between the normal and superdispersed emergence distributions may then explain why the testing for the optimality of male emergence patterns of butterflies in nature has yielded mixed results (Iwasa et al. 1983, Baughman et al. 1988, Sawada et al. 1997). Given a non-zero variation in female emergence within years, frequency-dependent selection acting on the optimal degree of protandry (Fig. 1), and stochastic variation in female emergence patterns among years (Iwasa and Haccou 1994), stabilizing selection in the vicinity of the long-term optimal protandry may be weak. Any pattern of variation about the optimum with non-zero variation would then persist in the population and, as indicated by our results, the specific pattern of the male emergence in nature may be far less important than variation in emergence, per se.

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