

Portrait of an Ephemeral Adult Stage: Egg Maturation, Oviposition, and Longevity of the Gall Midge *Rhopalomyia californica* (Diptera: Cecidomyiidae)

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Ann. Entomol. Soc. Am. 100(4): 549–561 (2007)

ABSTRACT Insect reproduction is typically spread out in time and space, making estimates of lifetime reproductive success quite difficult under natural field conditions. Such estimates are, however, critical to providing rigorous tests of theory for reproductive behavior. One approach to circumventing this problem is to study insects whose lifetime reproduction is compressed in space and time, and whose ovarian dynamics (oogenesis, oosorption, and oviposition) are simplified. We investigated the gall midge *Rhopalomyia californica* Felt (Diptera: Cecidomyiidae) as a possible candidate for an herbivore with such a simplified pattern of lifetime reproduction. We demonstrate here that *R. californica* matures all of its eggs before adult eclosion (it is strictly proovigenic), and it does not resorb eggs, even when deprived of oviposition sites. Observational and experimental studies in the field demonstrate that this midge typically completes its entire lifetime reproduction in a single day: females usually emerge at dawn, mate, and after a posteclosion period of resting, they initiate a sustained period of active oviposition during which most eggs are laid over a 4–5-h period. Mean longevity of adult females is very short, consistently <1 d and only 5–6 h on clear and warm days. Two key aspects of senescence are experimentally demonstrated: an increase in mortality rates and a decrease in oviposition rates with age. The life history traits associated with the ephemeral adult stage of *R. californica* are consistent with a syndrome that has been characterized recently for many proovigenic herbivorous and parasitic insects. Proovigenic species, including *R. californica*, may be particularly valuable as model systems for studies of lifetime reproductive success in nature.

KEY WORDS life history, proovigeny, oosorption, oviposition, senescence

Insect herbivores and parasitoids have been used extensively as model systems for developing theory in behavioral and evolutionary ecology, in part because they are relatively easy to study in the laboratory (Price 1980, Godfray 1994, Wajnberg 2006). Often, however, rigorous tests of theoretical predictions require estimates of reproductive costs and benefits in nature, rather than in the artificial laboratory environment. Such estimates have proven to be difficult to obtain (for reviews, see Casas 2000, Casas et al. 2004) for at least two reasons. First, reproduction by individual female herbivores and parasitoids is usually spread out over time and space, making it difficult to sum across an individual's lifetime. Second, many adult insects have relatively complex processes of ovarian dynamics, with mature egg inventories increasing through ongoing oogenesis, which may occur at highly variable rates (Papaj 2000, 2005), and with inventories decreasing through either oviposition or oosorption (Bell and Bohm 1975, Rivero-Lynch and Godfray 1997, Papaj 2000, Rosenheim et al. 2000,

Asplen and Byrne 2006). Ongoing oogenesis can be fueled by an array of foods available to ovipositing adults, including feeding on nectar, pollen, honeydew, fruit juices, and, for parasitoids, their hosts ("host feeding"; O'Brien et al. 2002; Casas et al. 2003, 2005). Oviposition and oosorption have very different implications for female fitness increments, despite producing identical changes in the oocyte inventory. These ovarian dynamics mean that for most insects it is not possible to estimate lifetime reproductive success from simple dissections. Point estimates of oviposition rates may be possible in some cases (e.g., Tatar 1991, Visser 1994, Casas et al. 2000), but only for insects that do not demonstrate senescence (accelerating mortality rates or decelerating oviposition rates with age; Tatar 2001) can point estimates easily be translated into lifetime estimates of reproduction.

One possible means of ameliorating these problems is to study insects that exhibit the traits that typify herbivores or parasitoids, but whose range of reproductive activity is compressed in time and space, making lifetime estimates more tractable. Insects with temporally compressed reproduction are, further-

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more, more likely to exhibit simplified ovarian dynamics. Both empirical and theoretical analyses suggest that very short-lived arthropods are more likely to be strictly proovigenic, with egg maturation occurring during the pupal stage rather than the adult stage. The ability to resorb mature oocytes also may often be lacking in species with short-lived adults (Jervis et al. 2005). When egg maturation and resorption are known to be absent, we can estimate realized individual lifetime reproduction from a simple dissection of females at the end of their lives (Jepsen et al. 2007).

The goal of this study was to develop a quantitative picture of the behavior, longevity, and oocyte dynamics (egg maturation, resorption, and oviposition) for the gall midge *Rhopalomyia californica* Felt (Diptera: Cecidomyiidae), to determine whether this species might be valuable as a model organism for studies of lifetime reproductive success in nature. We also sought to evaluate what we considered an unlikely possibility, namely, that there are no age-dependent increases in mortality rates or decreases in oviposition rates, to see whether point estimates of reproductive success could be projected directly to lifetime estimates. *R. californica* induces terminal multichamber bud galls on its sole host plant *Baccharis pilularis* De Candolle (Asteraceae), a dominant native shrub distributed along coastal California and Oregon, with some inland populations extending across California's Central Valley and into the foothills of the Sierra Nevada (Gagné and Boldt 1995). *R. californica* seemed to be a good candidate for several reasons. First, *R. californica* is a member of the family Cecidomyiidae, many of whose members are known to be extremely short-lived (Gagné 1989, 1994; Yukawa and Rohfritsch 2005). For example, several *Rhopalomyia* spp. associated with sagebrush, *Artemisia tridentata* Nutt., seem to have field longevity of <1 d, emerging at dawn and becoming difficult to find by mid-day (Jones et al. 1983). The family Cecidomyiidae includes some species whose egg maturation seems to occur gradually during the adult stage ("synovigeny"), including both gall-inducing herbivores (Pivnick and Labbé 1993) and predatory taxa (Havelka and Zemek 1999, Mo and Liu 2006). Nevertheless, most gall inducers are described as proovigenic (Miller and Borden 1984, Harris and Rose 1989, Hinz 1998, Harris et al. 2003, Yukawa and Rohfritsch 2005), although in most cases rigorous demonstrations are lacking. Oosorption has, to our knowledge, never been explored for members of the Cecidomyiidae. Second, we already know a good deal about the interactions of this midge with its sole host plant, *Baccharis pilularis* (Miller and Weis 1999, Latto and Briggs 1995, Rudgers and Whitney 2006), and its community of parasitoids (Doutt 1961; Force 1974; Ehler 1982; Hopper 1984; Ehler and Kinsey 1993; Briggs and Latto 2000, 2001; Darrouzet-Nardi et al. 2006; Langellotto et al. 2006). Third, although the reproductive ecology of *R. californica* has not been the focus of previous studies, early descriptions of the basic natural history of the midge (Tilden 1951) and insight gleaned from later work clearly indicate that this midge is very short-lived, with various authors

suggesting that female longevity is generally <2–3 d and that females are weak flyers (Hopper 1984, Ehler and Kinsey 1993, Palmer et al. 1993, Latto and Briggs 1995, Miller and Weis 1999, Briggs and Latto 2000). Tilden (1951) furthermore observed that the mouthparts and gut are reduced and may be nonfunctional, and no authors have recorded feeding behavior by the adults. Field experiments have demonstrated that most reproduction occurs within a few meters of the site of female emergence (Briggs and Latto 2000). *R. californica*, like many cecidomyiid midges, does seem, therefore, to concentrate its lifetime reproduction in a small time and space. Although nothing has been reported on egg maturation by this midge, or egg resorption for this or any other midge, the suggestion that adults may not feed makes it more likely that egg maturation might occur before adult eclosion (Jervis et al. 2005).

The specific questions that we addressed were as follows. What is the timing of *R. californica* reproduction on a seasonal and daily basis? Does *R. californica* mature or resorb eggs during the adult stage? How long does *R. californica* live in the field? What is the daily pattern of *R. californica* behavior in nature? Does *R. californica* feed during the adult stage? and Does *R. californica* show accelerating mortality rates or decelerating oviposition rates as they age (two aspects of senescence)?

Materials and Methods

Study Site. Our main study site was located just north of the University of California G. Ledyard Stebbins Cold Canyon Preserve and just east and south of the confluence of Cold Creek and Putah Creek (38° 30.71' N, 122° 5.76' W). The site, ≈50 by 200 m, was subject to both natural and anthropogenic disturbance, and it was characterized by a broken pine and oak overstory and a shrubby understory dominated by *Baccharis pilularis* and *Toxicodendron diversilobum* (Torr. & Gray Greene).

Phenology. Although coastal populations of *R. californica* reproduce actively year-round, inland populations like the population that we studied are associated with host plants that may cease vegetative growth during the dry and very hot summer; inland midge populations therefore seem to peak in density during the late winter and early spring, when rainfall triggers a flush of growth in their hosts (Doutt 1961, Ehler 1982, Ehler and Kinsey 1993; see also Palmer et al. 1993). We therefore conducted our studies during the late winter and spring. To describe the winter and spring occurrence of *R. californica* at the Cold Canyon site, we gathered three types of data. First, at irregular intervals from December to June, 2002–2006, we gathered samples ($n = 15–40$) of the terminal 5 cm of *B. pilularis* branches from across the study site. These terminals were returned to the laboratory and searched under a dissecting microscope to count all eggs and young first instars that had not yet left the exposed surface of the plant and crawled beneath bud scales.

Second, during the first midge generation of 2006 (22 February–21 April), we placed dry pan traps beneath *B. pilularis* to capture midges as they died and fell from the plant canopy. Pans were white plastic cafeteria trays (35 by 45 cm; NSF, Huntington Beach, CA) that we fitted with two layers of mesh (a thicker layer of plastic mesh under a finer metal window screening) in which the dead midges lodged securely. To avoid producing unnatural pools of water on the boards during rainy weather and to prevent any midges from being washed off the boards during heavy rain events, we drilled holes in the corners of the boards and covered them with fine mesh, so that the boards would drain. Because dead midges usually lodged on one of the two layers of mesh on the board surface, they were almost never found on the pan's screened drains. Trays were generally placed in the field late in the afternoon on Sundays, and then they were checked every 1–2 d, Monday–Friday. Midge captures on the pans are reported as number per 200 pan traps (the exact number of pans varied somewhat, but averaged ≈ 200). Pans were generally checked in the late afternoon; on occasions when pans were checked before 1200 hours, midge collections were assigned to the previous day, which was more likely to have been the day of their emergence. Midge captures were related to weather data gathered at a NOAA climatological station (Markley Cove, 38° 30' N 122° 07' W) located 3.5 km southwest of our field site.

Finally, to define the initiation of adult emergence during the second midge generation in 2006, we placed small polyester mesh bags around ≈ 55 newly developed (second generation) galls on 20–26 March. These bags were checked for newly emerged midges every 1–3 d.

Test for Egg Maturation or Resorption. We performed a manipulative experiment in the laboratory during June, 2002 to determine whether *R. californica* can mature or resorb eggs during the adult stage. We wanted our treatments to address two key possibilities. First, it is possible that *R. californica* can mature additional eggs as an adult, but that the ovaries are "full" at the time of their emergence, having reached their maximum capacity to store mature oocytes. Thus, a treatment in which females were able to oviposit, thereby freeing space in the ovaries, was needed to determine whether egg maturation would ensue. Second, it is possible that *R. californica* can resorb oocytes but perhaps only under conditions where oviposition substrates are lacking. Thus, a treatment in which *R. californica* are held for an extended period in the absence of host plant material was needed to determine if egg resorption would ensue.

Galls harboring nearly mature *R. californica* were collected in the field at Point Reyes National Seashore and returned to the laboratory where they were held singly for not > 7 d in 20-ml plastic vials at $25 \pm 1^\circ\text{C}$ and ambient photoperiod to await midge emergence. Vials were checked twice daily (at ≈ 0800 and 1700 hours), and emerging female midges assigned randomly to one of three treatments, each replicated 18–20 times, as follows. Treatment 1, emergence: newly emerged vir-

gin female midges were immediately placed in a freezer and held until dissection. Treatment 2, no oviposition substrate: newly emerged midges were moved singly into 20-ml plastic vials with a male, allowed to mate, and then they were held without access to any oviposition substrate. They were checked twice daily (0800 and 1700 hours), and, upon death, they were stored in the freezer until dissection. Treatment 3, oviposition substrate: newly emerged females were moved singly into 20-ml plastic vials with a male, allowed to mate, and then they were provided with an oviposition substrate, a field-collected 3-cm growing tip of *B. pilularis*, which was inserted into a water pik. Midges were again checked twice daily until the time of their death, and they were held in the freezer until dissected. Eggs laid on the *B. pilularis* tips were counted under a stereomicroscope. Occasionally, midges also laid eggs 1) in the vial or on the gall from which they emerged, or 2) on the vial in which they were held with or without host material. We counted all of these eggs laid before midge death, and we added these counts to the number of eggs remaining in the ovaries to estimate the total number of eggs matured.

Midges were held at -20°C for not > 5 d, and then they were dissected in a drop of water under a stereomicroscope to count all mature oocytes. For 12–15 females in each treatment, mature oocyte length for 10 haphazardly selected oocytes also was measured under a compound microscope by using an ocular micrometer. Females were then slide mounted, and the lengths of the mid- and hind tibiae were measured as indices of female size.

Longevity, Oviposition, and Activity Budgets: Field Cage Experiments. We conducted three experiments with small field cages to quantify midge longevity, oviposition, and activity budgets.

Experiment 1. The goal of this experiment, conducted 18–19 April, 2003, was to quantify the longevity of male and female midges. Galls were collected from a site 100 m west of the main study site during the afternoon of 17 April. Galls were held in small groups in 20-ml plastic vials fitted with a mesh-covered lid to provide ventilation. Vials were held in the field at the collection site, under the canopy of *B. pilularis*. Vials were checked hourly starting at 0500 hours to obtain newly emerged male and virgin female midges (any females that emerged in vials with males, and which might therefore have mated before we found them, were not used). Each newly emerged female was paired with a male and observed until mating occurred (virgin females mated readily, usually within a minute of exposure to males). Virgin males recently emerged from a gall other than the one from which the female had emerged were used when these were available, as this allowed us to measure male longevity as well as female longevity. When such males were not available, we used an older male that we reared or that was attracted from the surrounding midge population; in these cases, we did not attempt to estimate male longevity.

The pair of midges was then transferred to a cylin-

drical clear plastic cage (15 cm in height, 7 cm in diameter) whose top was covered with fine mesh and which bore a mesh-covered ventilation window on one side. The cage was placed over an intact (i.e., not detached from the plant) 10-cm growing tip of *B. pilularis* that we had searched to remove any predators or spider webbing, and the bottom of the cage sealed with a foam plug. Cages were then checked hourly to categorize each midge as alive or dead (midges were considered to be dead when they were immobile and not standing on their tarsi; midges in this condition were never observed to recover). Two of the experimental females continued to attract males from the surrounding population for the duration of the trial, and they never oviposited. We consider these to be instances of failed mating, and we excluded these replicates from our analyses. Once both midges were dead, the midges and the *B. pilularis* tip were returned to the laboratory. Midges were dissected to count mature eggs remaining in the ovaries, and hind tibia lengths were recorded as an index of body size. *Baccharis* tips were inspected under a stereomicroscope to count all eggs laid. Counts of eggs laid on *Baccharis* were somewhat inflated by eggs present on the tips before the cages were put in place, but this source of unexplained variance should not have been correlated with other variables of interest to us.

Experiment 2. The goals of this experiment, conducted 6–9 May 2003, were to quantify midge longevity and behavior as a function of age and to test whether the presence of sugar-rich food prolongs adult life. Methods were as described for experiment 1, with the following modifications. We categorized live midges as resting, active (including probing/ovipositing, walking, or flying), or moribund (still moving, but not standing on the tarsi). In half of the replicates, we affixed to the side of the cage a 5- by 10-mm strip of filter paper saturated with a 50% honey, 50% water solution. Cages were checked hourly for the first 15 h and then at less regular intervals over days 2–3. Time of death was assigned as the midpoint of the interval between checks. Ten *B. pilularis* tips collected in the study area on the day after the experiment was completed had no eggs present on them; thus, our counts of eggs laid by the experimental females should not have been inflated by previously laid eggs.

Experiment 3. The goals of this experiment were to quantify midge longevity, behavior as a function of age, and the time course of oviposition by midges. Methods were as described for experiment 1, with the following modifications. The experiment was conducted 1–28 April 2004, with replicates set up on four different dates (=blocks). Because galls were not abundant at the study site, we collected galls at a site 37 km south of our primary study site, just west of Mare Island, Solano County. Galls were held in the laboratory for 1–3 d, and females that emerged at dawn were mated and brought to the main field site. Females only were placed in the standard cage with an 8-cm freshly cut tip of *B. pilularis* and held on the ground under a *B. pilularis* canopy. We obtained a supply of fresh *B. pilularis* tips that were free of midge eggs by placing

mesh sleeves over *B. pilularis* branches at our study site at least 2 wk before the tips were used; the 2-wk period allowed previously laid eggs to hatch or to reach late stages of embryonic development, which were readily distinguishable from freshly laid eggs. Cages were checked at hourly or half-hourly intervals to record midge behavior and replace the *B. pilularis* tip with a fresh tip. All *B. pilularis* tips were returned to the laboratory to count the number of eggs laid. Female midges were collected at the time of their death (or, rarely, when they were moribund and no longer on the plant; see Results), and held in a freezer for not >2 d until they were dissected and measured.

Statistical Analyses. Longevity data were evaluated using survival analysis (Kleinbaum and Klein 2005). Kaplan–Meier survival estimates were calculated with JMP 6.0 (SAS Institute, Cary, NC). Weibull distributions (proportion of individual surviving to age $x = p(x) = e^{-(x/\alpha)^\beta}$) were found to fit the data well; Weibull parameter estimates and 95% confidence intervals (CI) are reported. Shape parameters, β , whose values are >1.0 indicate that the hazard function (the instantaneous risk of mortality) increases with age (i.e., senescence). Log-rank tests were used to compare longevities across groups. Cox's proportional hazards model was used to evaluate the influences of female size and total egg production on female longevity. Nonparametric Kruskal–Wallis tests were used to assess shifts in female midge activity over their adult lives, with multiple observations for each female during a given time period collapsed to a single datum (e.g., proportion of observations during which the midge was active) to avoid pseudoreplication. To test for age-dependent changes in oviposition rate for midges known to still have eggs remaining in their ovaries, a linear regression of eggs laid per time period (30 or 60 min, and excluding time periods during which the midge transitioned from the posteclosion rest period to active oviposition, or died) versus hours after the initiation of oviposition was performed for each midge, and the resulting slope parameter used as an index of reproductive senescence. To plot mean oviposition rate versus midge age at the population level, oviposition rates in time intervals during which midges either 1) made the transition from resting to ovipositing or 2) died, were calculated by assuming that the time available to lay the eggs was half of the total time interval.

Activity Budgets: Observations of Freely Foraging Females. We studied midges in the field to document their daily patterns of emergence and the behavior of freely foraging individuals.

Timing of Emergence. To document the timing of male and female midge emergence, we collected galls, held them in ventilated vials in the field, and we monitored them for emergence. On the afternoon of 17 April 2003, we collected a sample of galls from *B. pilularis* growing \approx 100 m west of our main study site. Galls were placed in groups of one to three in 20-ml plastic vials fitted with a mesh-covered lid and held under the canopy of *B. pilularis* at the collection site. Beginning at 0500 hours on 18 April the galls were checked hourly for the next 17 h to record the timing

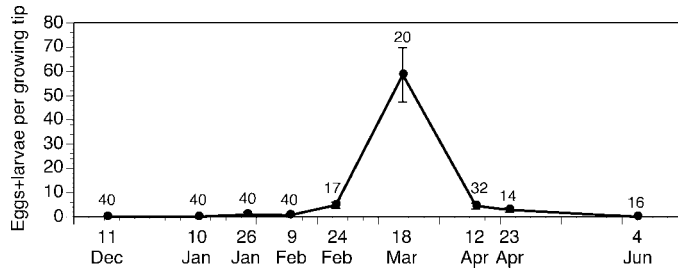


Fig. 1. Mean \pm SE number of *R. californica* eggs plus newly hatched first instars found per growing tip of *B. pilularis* during winter and spring, 2003 and 2004. Sample sizes are shown above each data point.

of midge emergence. On the afternoon of 5 May 2003, we again collected galls (≈ 500) from the same site, and beginning at 0400 hours on 6 May, we checked them hourly (or every 2 h between 0000 and 0400 hours) to record the timing of midge emergence over the next 2 d.

Behavior. We conducted focal observations at the main study site of female midge behavior in the field to quantify daily activity budgets. We observed midges at all times during the day when we could find them in the field. Midges ($n = 182$) were observed between 12 March and 12 May 2003, 9 February and 23 April 2004, 7 March and 11 April 2005, and 21 February and 19 April 2006. *B. pilularis* plants were searched for midges, and the first female discovered was monitored continuously by one to two observers for as long as possible, with behavior recorded on a hand-held computer operating behavioral event recording software. Newly emerged females resting on the host plant were inconspicuous; thus, while looking for midges, we also searched carefully near mature galls from which midges might have emerged recently. Tilden (1951) noted that female midge appearance changes as they deposit their inventory of bright orange eggs. Thus, at the start of the observation, we scored females for abdomen girth (on a 0–4 scale: 0, very thin to 4, very distended with eggs) and for whether the orange eggs were visible through the wall of the abdomen (on a 0–2 scale: 0, eggs not visible, abdomen looks brown; 1, eggs faintly visible; and 2, orange eggs clearly visible through abdomen wall, abdomen looks orange). We categorized behaviors as follows: rest (midge immobile, abdomen not contacting the substrate), walk (midge walking with the abdomen not contacting the substrate), fly (wings beating), probe/oviposit (midge moving or stationary with the abdomen curved downward and in contact with the substrate; it was not easy to distinguish probing from ovipositing, and midges often alternated repeatedly between these two behaviors), and out of view (midge hidden by plant structures). We also categorized the midge's location as being either on the *B. pilularis* host plant, on the ground, or on any other plant. Matings also were recorded. The mean duration of an observation was 21.8 min (total observation time: 66.2 h). For consistency of time records, we did not advance our research clocks with the onset of daylight savings. For all statistical analyses a single observation is taken as the unit

of independent replication. To prepare figures, however, very long observations of females that were resting in the early morning hours were broken into hourly increments.

Results

Phenology. Samples of *B. pilularis* tips taken during 2004 revealed that peak egg laying by the first generation of *R. californica* occurred in March (Fig. 1). Tip samples taken during March 2003, 2005, and 2006 suggested that this pulse of egg laying could be observed reliably at around this time (mean eggs plus larvae observed in tip samples: 5 March 2003, 9.4 ± 2.0 [$n = 40$]; 15 March 2005, 67.0 ± 10.8 [$n = 3$]; 16 March 2006, 26.3 ± 8.1 [$n = 2$]). Also consistent with the pattern recorded in 2004, an earlier sample taken on 21 February 2006 failed to reveal many eggs/larvae (mean = 0.1 ± 0.1 , $n = 20$). Although our sampling during 2004 did not document egg laying by a second generation of *R. californica* (our sampling intervals may have been too infrequent to detect a narrow peak of oviposition), tip samples taken in 2002 did reveal a very small peak of egg laying in early May (mean eggs + larvae per tip: 22 April, 2.0 ± 0.7 [$n = 31$], 2 May, 3.8 ± 1.3 [$n = 3$], 28 May, 0.5 ± 0.2 [$n = 40$]). Informal observations made during 2006, which was a year with unusually heavy late spring rainfall, suggested that the second generation was large that year and probably substantially larger than the first generation; the first adult midges emerged from our sample of bagged second generation galls on 19 and 21 April, ≈ 2 mo after the onset of emergence of the first generation. Thus, there seems to be a consistent early spring generation in March and a more variable late spring generation occurring ≈ 2 mo later whose size may be linked to spring rainfall.

Informal observations made during 2002–2005 suggested that midge emergence occurred primarily during periods of dry weather. Pan trap catches in 2006 confirmed this suggestion: peaks of midge emergence occurred between periods of rain (Fig. 2). During the main period of midge emergence (23 February–16 March), there was significantly less rainfall on days for which two or more female midges were collected (mean rainfall = 0.15 ± 0.11 cm [$n = 8$]) than on days for which ≤ 1 female midge was collected (mean rainfall = 1.20 ± 0.57 cm [$n = 7$]; $\chi^2 = 3.3$, $P = 0.03$;

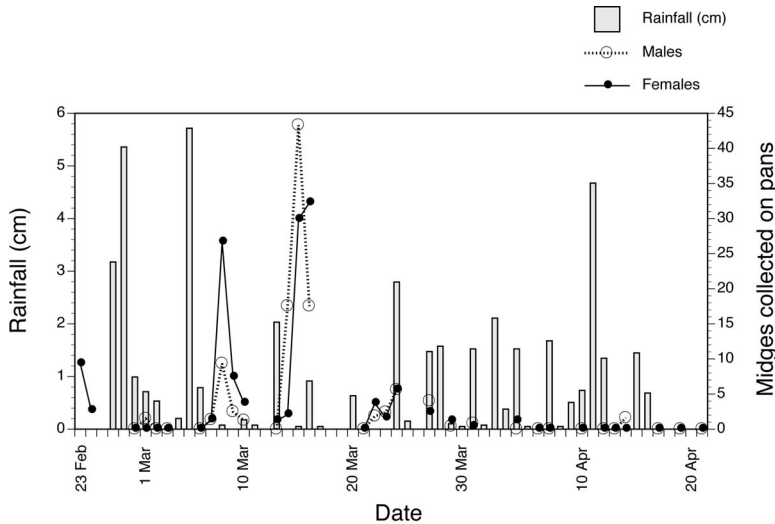


Fig. 2. Influence of rainfall on pan trap catches of male and female *R. californica* during the first generation of 2006 (23 February–21 April).

one-tailed Wilcoxon test). However, neither male nor female midge pan trap captures was correlated with daily temperature maxima or minima, or with the temperature near the time of emergence (0800 hours) ($P > 0.10$ in all cases). Thus, midges seem to avoid emergence on rainy days, but they seem to use a cue other than temperature to do so.

Test for Egg Maturation or Resorption. The treatments succeeded in producing different opportunities for midges to mature and resorb eggs. Mean midge longevities in the three treatments differed significantly (midges were killed immediately upon emergence in the emergence treatment, and they lived for 1.6 ± 0.2 d in the no oviposition substrate and 2.6 ± 0.2 d in oviposition substrate treatments; $F_{2, 55} = 56.2$; $P < 0.0001$). As intended, female oviposition was increased by providing the *Baccharis* tip as an oviposition substrate (mean number of eggs laid was 8.1 ± 7.3 [$n = 20$] in the emergence treatment, 14.5 ± 10.8 [$n = 20$] in the no oviposition substrate treatment, and 60.4 ± 21.8 [$n = 18$] in the oviposition substrate provided treatment; $F_{2, 55} = 4.0$; $P = 0.02$).

We found no evidence that female midges mature or resorb eggs during the adult stage (Fig. 3). The total number of eggs that females laid or retained in their ovaries was positively correlated with female size (analysis of covariance [ANCOVA], effect for hind tibia length; $F_{1, 54} = 26.3$; $P < 0.0001$), but the size-fecundity relationships did not differ significantly across treatments ($F_{2, 54} = 1.5$; $P = 0.23$; ANCOVA). The mean total number of eggs laid or retained in the ovaries was 259.5 ± 20.9 in the emergence treatment, 232.4 ± 13.9 in the no oviposition substrate treatment, and 275.6 ± 21.2 in the oviposition substrate treatment. The mean length of oocytes also was not influenced by either treatment ($F_{2, 34} = 0.6$; $P = 0.54$; ANCOVA) or midge hind tibia length ($F_{1, 34} = 0.5$; $P = 0.49$). Because some females from all treatments ovi-

posited on the vials or the gall from which they emerged, we also asked whether females that oviposited at any point during the experiment differed in their total egg production from females who never oviposited; the answer was negative ($F_{1, 56} = 0.5$; $P = 0.48$; mean number of eggs 259.4 ± 12.0 for females who did not lay eggs and 240.4 ± 26.4 for females who did lay eggs; ANCOVA).

During the dissections, we found no visual evidence for either egg maturation or resorption. Eggs being resorbed are often conspicuous because of their irregular shapes (Bell and Bohm 1975); such eggs were

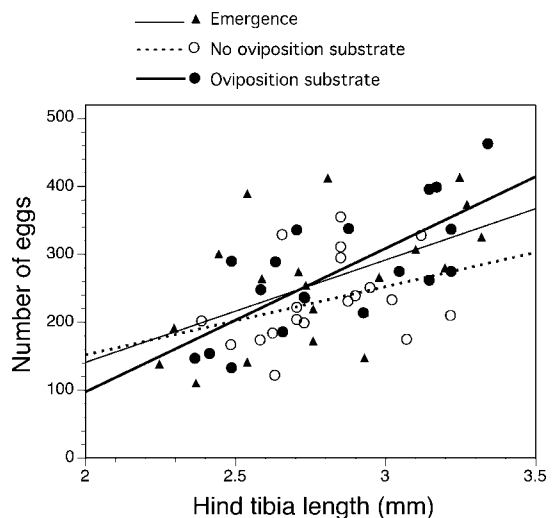


Fig. 3. Total number of eggs matured by female *R. californica* when dissected at emergence (emergence), when held with no oviposition substrates until death (no oviposition substrate), and when held with a tip of their host plant *B. pilularis* until death (oviposition substrate).

never observed in our study. Immature oocytes are usually readily identifiable by their smaller size, the presence of associated nurse cells, and the lack of a chorion (Chapman 1998); again, none of these signs were observed. Variation in oocyte length was generally small; among the 10 eggs measured per female, the maximum length difference between the longest and shortest eggs averaged only $9.8 \pm 0.6\%$ of the mean length of the mature oocytes ($n = 39$), and this proportional measurement of egg size variation did not vary across treatment ($F_{2,36} = 2.3$; $P = 0.11$). Each of these observations is consistent with the interpretation that female midges emerge with their full complement of eggs already matured (i.e., they are strictly proovigenic) and that they do not resorb eggs during the adult stage.

Longevity, Oviposition, and Activity Budgets: Field Cage Experiments. *Experiment 1.* Adult females lived longer than adult males, but both sexes had short longevities (mean longevity: females = 13.01 ± 1.96 h [median = 12.00, $n = 12$]; males = 5.83 ± 0.37 h [median = 5.83; $n = 5$]; $\chi^2 = 9.5$, $df = 1$, $P = 0.0021$; log-rank test). Weibull functions fitted to the longevity data suggested that the rate of mortality increased with age in both male and female midges (i.e., β parameters were significantly >1.0 : for males, best fit parameters: $\alpha = 6.12$ [95% CI: 5.47–6.81], $\beta = 10.9$ [95% CI: 4.51–21.0]; for females, $\alpha = 14.8$ [95% CI: 10.8–19.8], $\beta = 2.14$ [95% CI: 1.33–3.15]). Female longevity was not correlated with either female size (hind tibia length [millimeters]: proportional hazards parameter = 17.8 ± 15.4 [SE], $\chi^2 = 1.5$, $P = 0.23$) or total number of eggs matured (proportional hazards parameter = 0.0040 ± 0.0039 , $\chi^2 = 1.2$, $P = 0.28$).

Experiment 2. Midges were never observed to feed on the honey solution provided, and mean female longevity was not influenced by the presence of honey (mean longevity, honey present = 22.6 ± 3.5 h [$n = 12$]; honey absent = 17.5 ± 2.6 h [$n = 11$]; $\chi^2 = 0.9$, $df = 1$, $P = 0.34$; log-rank test). We therefore combined the honey \pm treatments in subsequent analyses. As was observed in experiment 1, females lived longer than males (mean longevity: females = 20.17 ± 2.23 h [median = 15.0, $n = 23$]; males = 9.25 ± 1.03 h [median = 11.0; $n = 4$]; $\chi^2 = 22.9$, $df = 1$, $P < 0.0001$; log-rank test). Fitted Weibull functions again suggested that the rate of mortality increased with age for both males and females (males: $\alpha = 9.99$ [95% CI: 7.94–12.45], $\beta = 6.23$ [95% CI: 2.23–12.97]; for females: $\alpha = 22.90$ [95% CI: 18.31–28.32], $\beta = 2.07$ [95% CI: 1.49–2.71]). In a model that included a term for the effect of the day on which the replicate was set up, female longevity was significantly greater for larger females (hind tibia length [millimeters]: proportional hazards parameter = -28.6 ± 13.0 , $\chi^2 = 5.0$, $P = 0.026$) but not significantly so for females that matured more eggs (proportional hazards parameter = -0.0054 ± 0.0033 , $\chi^2 = 2.6$, $P = 0.10$).

Female midges exhibited clear shifts in behavior over time (Fig. 4; $\chi^2 = 30.9$, $df = 3$, $P < 0.0001$; Kruskal-Wallis test for proportion of observations during which midges were active). Immediately after

emergence from the pupa in the morning (all midges used in the experiment emerged between 0600 and 0900 h), females spent most of their time ($82.6 \pm 6.0\%$ of all observations, $n = 23$ females) resting. Two observations suggest that this resting period, which averaged 2.91 ± 0.24 h, is likely to be associated with maturational processes after eclosion (e.g., hardening of the cuticle), rather than a response to cooler morning temperatures: first, there was no correlation between the duration of the rest period and the time of emergence ($r = 0.13$, $df = 21$, $P = 0.54$; we would have expected longer rests for earlier-emerging females if the rest was imposed by cool morning temperatures); and, second, we did not see a rest period during the early morning on the second day of the midges' lives (Fig. 4). After the posteclosion rest, we observed a period of intense activity, extending from ≈ 3 h posteclosion until 2200 hours (well after sunset), during which all behaviors were apparently linked to locating and evaluating oviposition sites, and laying eggs (walking, flying, probing, ovipositing; $71.5 \pm 3.9\%$ of all observations, $n = 23$ females). From 2300 hours until 0500 hours, the next morning, midge activity decreased, with midges resting in $76.7 \pm 11.2\%$ of all observations ($n = 10$ females). Finally, the few midges ($n = 5$) that lived long enough to reach the second morning of their adult lives again showed an increase in activity (midges active during $73.8 \pm 13.4\%$ of all observations). Each of these transitions in activity level was significant, as judged by a Wilcoxon test ($P < 0.03$ in all cases).

Experiment 3. Female midge longevity was again very short (median longevity across all four blocks = 6.08 h; because six of the 26 midges were collected when they were moribund, rather than dead, these censored observations make the calculated mean longevity [6.34 h] slightly biased). There were significant differences in survival across the four blocks of replicates ($\chi^2 = 32.1$, $P < 0.0001$); Weibull functions were therefore fitted for each block separately. In each case, the fitted β parameters were significantly >1.0 , suggesting that the rate of mortality increased with midge age (data not shown). A proportional hazards model that included block as a grouping factor suggested that female longevity was not correlated with either female size (hind tibia length [millimeters]: proportional hazards parameter = -18.1 ± 15.9 [SE], $\chi^2 = 1.3$, $P = 0.25$) or total number of eggs matured (proportional hazards parameter = 0.00093 ± 0.0088 , $\chi^2 = 1.2$, $P = 0.27$).

Female midges rested after emergence and before initiating oviposition (mean rest duration = 2.94 ± 0.26 h; $n = 12$). After this rest, females entered a period of intense and sustained oviposition activity (Fig. 5). Note that our behavioral observations were made at the start of the hourly interval, so that females had not yet begun ovipositing when checked at the start of the hour during which they eventually became active. Egg laying rates declined continuously with female age (mean slope value from linear regression of eggs laid versus time [hours] after the initiation of oviposition for individual females, excluding the time periods dur-

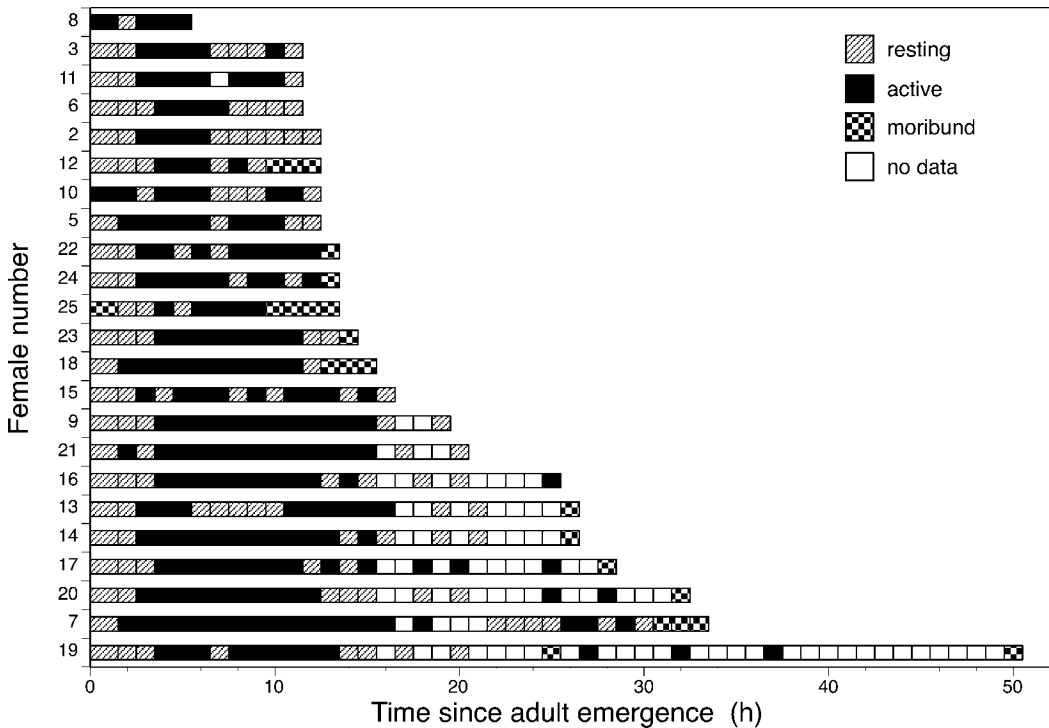


Fig. 4. Activity budget of female *R. californica* midges as a function of time since female eclosion from the pupa. Midges were observed in small enclosures in the field. Each horizontal bar shows the time course of behavior expressed by a single female.

ing which females began oviposition or died: -39.2 ± 5.2 , $P < 0.0001$; signed-rank test that slope is significantly negative; $n = 25$). One contributor to this decline was that females simply ran out of eggs to lay (females died with an average of only 21.6 ± 7.7 eggs remaining in their ovaries; nine of 25 females laid all their eggs). Interestingly, four of the nine females who were revealed by our subsequent dissections to have exhausted their egg supply were still observed to express probing/oviposition behavior after they had laid their last available egg. To test if the rate of oviposition declined even for females who had not exhausted their egg supply, we again regressed oviposition rate versus time for individual midges, but now we excluded the final time period during which oviposition occurred and all subsequent periods. These regressions were on average still significantly negative (mean slope value = -31.8 ± 19.3 , $P = 0.04$; signed-rank test that slope is significantly negative, $n = 18$), suggesting that oviposition rates declined even before running out of eggs. The decline in oviposition rates with midge age is the second manifestation of senescence that we have observed for *R. californica*.

Longevity and Daily Temperature. We observed substantial variation in female midge longevity across days in our field trials (experiments 1–3). Not surprisingly, this variation was strongly correlated with daily temperature maxima on the days when the trials were set up (Fig. 6; $\rho = -0.96$, $P = 0.0005$; Spearman's nonparametric rank correlation). The data also sug-

gest that longevity may have been particularly extended on cloudy days.

Activity Budgets: Observations of Freely Foraging Females. Timing of Emergence. For the galls collected on 17 April 2003, 10 males and two females had emerged between the time of our setup of the galls at 1700 hours on 17 April and our first check for emergence at 0500 hours on 18 April. We then observed two periods of midge emergence over the next 17 h: one period during the early morning (0530–0900 hours), when seven males and 14 females emerged, and the second period during the early evening (1800–2200 hours), when 12 males and six females emerged. For the galls collected on 5 May 2003, nine males and 0 females had emerged between setup of the galls at 1700 hours on 5 May, and our first check for emergence at 0400 hours on 6 May. Over the next 2 d, we again observed two windows of emergence (Fig. 7): a male-dominated emergence during the evening (1900–2300 hours) and a female-dominated emergence during the morning (0200–0800 hours). Thus, our combined data suggest two windows of emergence activity, with male emergence concentrated in the evening (45/59 = 76.3%; $\chi^2 = 8.8$, $P = 0.003$) and female emergence concentrated in the morning (43/52 = 82.7%; $\chi^2 = 12.8$, $P = 0.0003$).

Behavior. We never observed midges to feed or drink in the field. We observed only a single mating event; it seems likely, then, that midges are mated very soon after emergence from the pupal case (and before

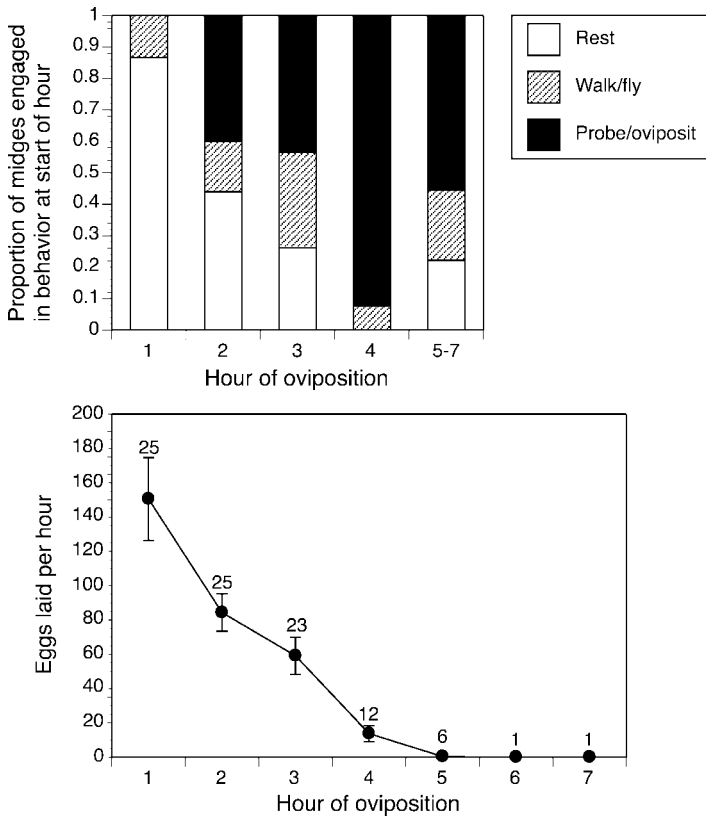


Fig. 5. Changes in female *R. californica* behavior (top panel) and oviposition rate (mean \pm SE; bottom) after the initiation of oviposition. For hour intervals during which midges either 1) transitioned from the posteclosion rest to active oviposition behavior, or 2) died, we calculated oviposition rates assuming that half of the hour was available for oviposition. Sample sizes for each time interval are shown above the data points in the bottom panel.

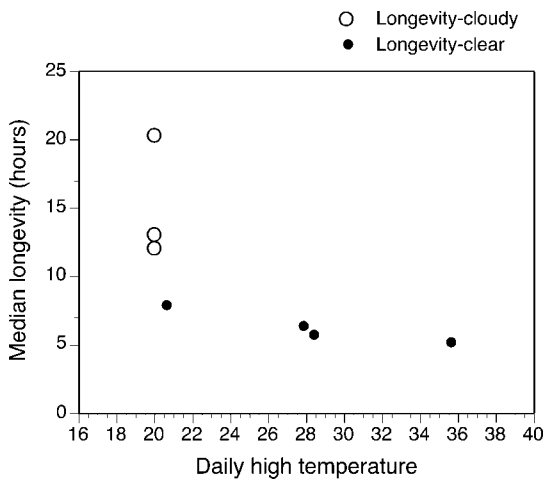


Fig. 6. Influence of daily temperature maxima ($^{\circ}$ C) on female midge longevity. Each data point represents the median female midge longevity observed for a cohort of midges initiated on a different date in the field cage experiments. Longevity seemed to be particularly extended on days with cloud cover. Open circles, cloud cover present; filled circles, cloud cover absent.

we could discover them to initiate observations), and they are not receptive to mating thereafter. Midges did not forage extensively before initiating oviposition: females that were observed making the transition from posteclosion rest to active oviposition spent on average only 1.8 ± 1.0 s flying (10 of 14 did not fly at all) and only 30.0 ± 7.6 s walking. Our observations also suggest that midges spend nearly all their time on their host plant, *B. pilularis*: for 180 of the 182 observed females, 100% of their time in contact with any substrate was spent on *B. pilularis*, with the two remaining females spending some time on the ground below *B. pilularis*.

Three trends were readily apparent in midge appearance and behavior over the course of the day. First, midge abdomens were heavily distended with eggs early in the morning and gradually thinned over the course of the day (linear regression of abdomen girth score on observation time: $r = -0.48$, $P < 0.0001$; $n = 124$) (Fig. 8). Second, eggs in the ovaries imparted an orange appearance to midge abdomens for most of the day, the color being lost only in the afternoon (linear regression of abdomen color score on observation time: $r = -0.34$, $P = 0.0002$; $n = 115$) (Fig. 8), when egg loads were heavily depleted (unpublished

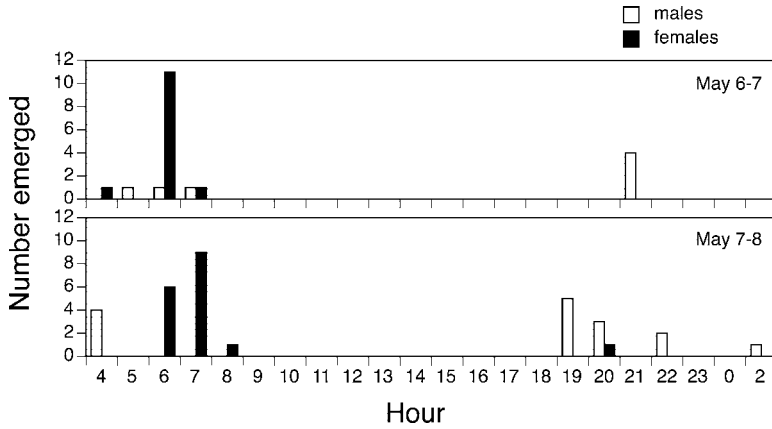


Fig. 7. Diurnal pattern of male and female *R. californica* emergence in the field, 6-8 May 2003. Newly emerged midges found at checks at the beginning of each hour were assigned emergence times during the preceding hourly interval (or 2-h interval for the one male midge that was found at 0400 hours on 8 May).

data). Third, consistent with our observations of midge behavior in small field enclosures, midges observed foraging freely in the field showed a clear shift

from resting during the early morning to active probing and oviposition during the remainder of the day (linear regressions of observation time on proportion

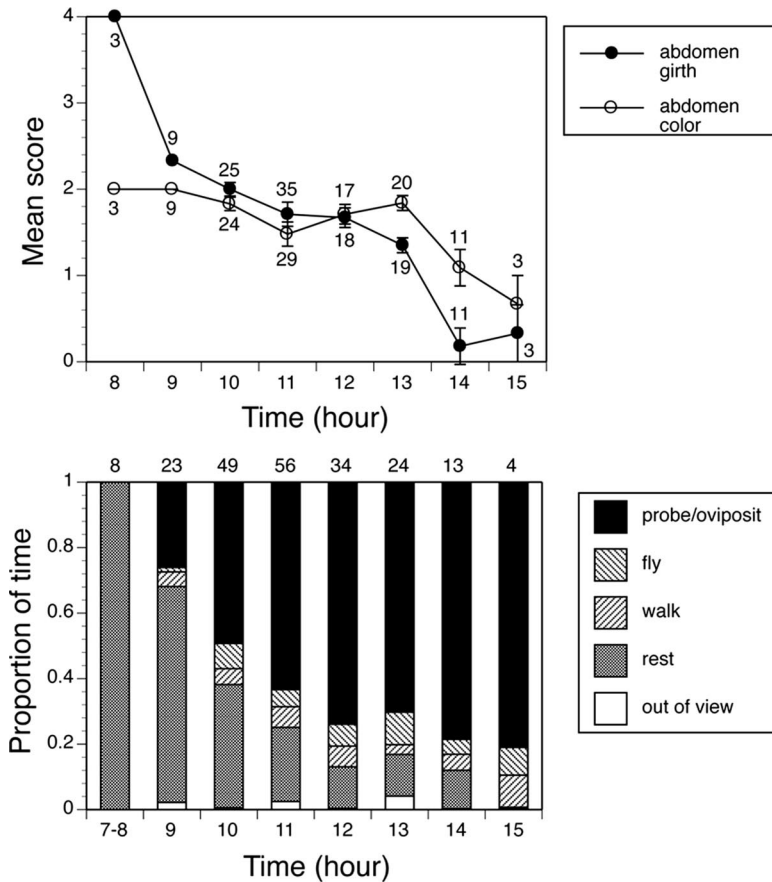


Fig. 8. Focal observations of freely foraging adult female midges in the field. Top, mean \pm SE score for abdomen girth (0, very thin to 4, heavily distended with eggs) and for abdomen color (0, brown; 2, orange) over the course of midge activity during the day. Numbers above and below the means are the numbers of replicate observations. Bottom, daily midge activity budget. Numbers above the histogram bars are the number of observations.

of time spent resting: $r = -0.43$, $P < 0.0001$; $n = 182$; and proportion of time spent probing/ovipositing on observation time: $r = 0.40$, $P < 0.0001$; $n = 182$).

Discussion

Recent comparative analyses have demonstrated that the remarkable diversity of life history traits exhibited by insects can be organized by arraying species along a continuum described by the "ovigeny index," defined as the fraction of the total lifetime complement of eggs that is mature at the time of adult eclosion (Jervis et al. 2001, 2003, 2005, 2007; also see Flanders 1950). Our study demonstrates that the gall midge *R. californica* occupies one end of this ovigeny continuum: 100% of the lifetime complement of eggs is mature at the time of female eclosion. Thus, this species has an ovigeny index of 1.0 (it is strictly proovigenic). As shown by Jervis et al. (2001, 2003, 2005, 2007), proovigeny, or equivalently, high values of the ovigeny index are strongly associated with several other life history traits for both parasitic (Hymenoptera) and herbivorous (Lepidoptera and Trichoptera) insects, generating a syndrome of life history traits that is mirrored in our results for *R. californica*, as follows.

First, proovigeny is generally associated with reduced dependency on feeding by the adult to support reproduction and adult longevity. Our study has confirmed both experimentally and observationally that *R. californica* is strictly nonfeeding in the adult stage. This confirms the earlier suggestion that the mouthparts and gut of this midge, which are reduced, may not function in feeding (Tilden 1951; also see Jones et al. 1983).

Second, proovigeny is generally associated with reduced adult longevities. This is true to an extreme degree for *R. californica*, for whom mean adult female longevity is consistently < 1 d, and as little as 5–6 h when emergence occurs on warm spring days. Because most female emergence occurs at dawn, under natural, unmanipulated field conditions the whole process of aging and senescence plays out over the course of a single day of intense foraging and oviposition activity. The extremely short longevity of *R. californica* is an extreme expression of an underlying trade-off between reproduction and survival, a core element of life history theory (Roff 2002).

Third, proovigeny is generally associated with a reduced ability to reallocate resources from oocytes to somatic maintenance through the resorption of eggs. Our results suggest that *R. californica* is unable to resorb eggs, even when deprived of suitable oviposition sites. This result is not surprising when viewed against the backdrop of other aspects of *R. californica*'s biology, and indeed it is difficult to imagine a scenario in which oosorption would enhance reproductive success in this gall midge. Oosorption is generally a slow process, during which oviposition cannot occur (Jervis and Kidd 1986). Oosorption is therefore usually expressed when there is a transient pulse of nutrient stress and a lack of suitable oviposition sites; in these cases, an adult female may resorb some or all of

the mature oocytes to sustain herself until she can locate an environment with ample food and oviposition sites, when egg maturation is reinitiated (Bell and Bohm 1975, Jervis and Kidd 1986, Papaj 2000). For the gall midge *R. californica*, however, the female's life generally begins on the natal host plant, which is a long-lived perennial. Thus, oviposition sites are immediately available on a host plant that was, at least previously, suitable for immature development. Furthermore, with a nonfeeding adult stage, there is no question of a reversible pulse of nutrient stress: the nutrient budget of a female is independent of external environmental conditions. Under these constraints, any delay in reproduction is likely to be selected against, because total metabolic reserves can only decline, and eggs that are resorbed can never be replaced. Finally, unless oosorption were to produce dramatic increases in female longevity, there would seem to be little opportunity for adult female midges to wait long enough for environmental conditions to improve in a significant way.

Fourth, proovigeny is generally associated with low adult mobility and with a habit of laying eggs in clutches, rather than singly, which reduces the amount of movement that is required to find suitable oviposition sites. Briggs and Latto (2000) have indeed demonstrated that *R. californica* move very little over their lives, and although eggs may be laid singly or in small clusters, the total number of eggs laid per *B. pilularis* tip can be quite high (Ehler and Kinsey 1993, Latto and Briggs 1995, Miller and Weis 1999, Rudgers and Whitney 2006). Although we did not quantify net displacement during our field observations, our informal observations are consistent with the view that these midges are relatively sedentary, with most flights being short and within a single host plant.

Finally, proovigeny is generally associated with broad host plant ranges for herbivorous insects, which should again reduce the difficulty of locating suitable oviposition sites. *R. californica* is, however, a strict specialist, reproducing only on *B. pilularis* across its entire range; in this one regard only, *R. californica* diverges from the syndrome of life history traits associated with strict proovigeny. Our observations, however, suggest an explanation. Because the midge pupates right on its perennial host plant, extensive foraging is not required to locate oviposition sites. Most females that we observed at the start of their oviposition activity chose their host plant by default, by using the host plant individual on which they had developed themselves, and demonstrated only minimal movement through walking before ovipositing. Furthermore, *B. pilularis* is often a dominant shrub in the habitats where it occurs, making it likely that other host plant individuals are to be found nearby. Specialization does not seem to mandate extensive search for hosts involving energetically demanding flight in *R. californica*.

We documented two expressions of senescence in *R. californica*: mortality rates that increased with age, and oviposition rates that decreased with age. Because adult *R. californica* do not feed, metabolic reserves

needed to support basic metabolic processes are eventually exhausted, leading to escalating mortality; similar acceleration of mortality in nonfeeding adult insects has been recorded for mayflies (Carey 2002). Strict proovigyny similarly sets the stage for reproductive senescence, in large part because the finite and nonrenewable egg supply is exhausted as oviposition progresses. Decelerating oviposition rates in *R. californica* mean that it is not possible to use point-estimates of oviposition rates (e.g., the number of eggs deposited during a short focal observation in the field) to estimate lifetime reproductive success; direct measures of lifetime reproduction are needed to integrate across the changing time course of midge oviposition.

Given the ephemerality of adult *R. californica*, selection should act on midges to avoid emergence on days when heavy rain, or perhaps wind, would preclude oviposition. We observed that midge emergence is reduced on days with heavy rainfall, with pulses of female emergence occurring 1–2 d after heavy rains. Still unresolved is what cues midges can detect as pupae still residing inside the gall that would allow them to conduct this short-term weather forecasting, because temperature did not seem to play any role.

R. californica displays life history traits, including strict proovigyny, nonfeeding adults, and very short adult longevities, that seem extreme when viewed against insect life histories in general, but which may actually be widespread within the family Cecidomyiidae (Gagné 1989, 1994; Yukawa and Rohfritsch 2005). Although this study is, to our knowledge, the first to investigate oosorption in a gall midge, it seems likely that this trait also may be common among midges with nonfeeding and ephemeral adult stages. Thus, *R. californica*, and perhaps many other species within this diverse and widespread family, seem to have the temporally and spatially compressed reproduction that should make them ideal subjects for studies attempting to understand determinants of lifetime reproductive behavior in nature.

Acknowledgments

We thank Marisa Alcorta, Claire Connolly, Elizabeth Frost, Khanh Nguyen, and Andy Zink for assistance in the field. We also thank Shane Waddell and Shorty Boucher for making it possible to conduct the study with the protections afforded to the University of California Natural Reserve System. The manuscript was substantially improved by comments from Mark Jervis, Ken Spence, and an anonymous reviewer. This research was supported in part by a grant from the USDA-NRCCG award 2001-35316-11013. The manuscript was written while J.A.R. was supported in part by a Lady Davis Visiting Professorship, Hebrew University of Jerusalem, Israel.

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Received 15 March 2007; accepted 18 April 2007.