Ovarian development and lipid reserves are affected by mating delays in three species of *Anthocoris* (Hemiptera: Anthocoridae)

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Abstract—Mating is necessary to bring about ovarian maturation in females of Anthocoridae and related taxa (Cimicidae). The objectives of this study were to determine how forced delays in mating affect extent and rate of oocyte development, duration of the preoviposition period, and levels of lipid reserves in three species of *Anthocoris*. Extent of oocyte development by unmated females differed among the three species. In unmated *A. tomentosus*, the basal oocyte failed to show any increase in size with increasing female age, whereas oocytes in unmated *A. nemoralis* and *A. whitei* exhibited some growth beginning 2 days after eclosion. One consequence of these differences among species is that a forced delay in mating (of 3 or 10 days) had less of an effect on *A. whitei* and *A. nemoralis* than on *A. tomentosus*, in terms of the length of the preoviposition period measured from the time of mating. Mated females of *A. nemoralis* and *A. whitei* grew larger oocytes than unmated females within 2 days of mating, whereas the same phenomenon took 4 days in *A. tomentosus*. Embryos became visible in the eggs of mated *A. nemoralis* and *A. whitei* 2–3 days after mating, compared with 5 days after mating for *A. tomentosus*. Mature eggs with egg caps were visible within 3, 4, and 6 days after mating for *A. nemoralis*, *A. whitei*, and *A. tomentosus*, respectively. In all three species, unmated females 10 days after eclosion had significantly higher levels of lipids allocated to nonreproductive tissues than similarly aged females that had been mated on the day of eclosion, suggesting that there was a trade-off between allocation of resources to eggs and allocation to somatic reserves.

Résumé—L'accouplement est requis pour la maturation ovarienne chez les femelles d'Anthocoridae et des taxons apparentés (Cimicidae). Les objectifs de notre étude sont de déterminer comment des délais forcés de l'accouplement affectent le degré et le taux de maturation de l'oocyte, la durée de la période de pré-ponte et les concentrations de lipides chez trois espèces d'*Anthocoris*. Le degré de maturation des oocytes chez les femelles non accouplées varie chez les trois espèces. L'oocyte de base chez des femelles d'*A. tomentosus* non accouplées ne montre aucun accroissement en taille en fonction de l'âge de la femelle, alors que ceux de femelles non accouplées d'*A. nemoralis* et d'*A. whitei* montrent une certaine croissance commençant 2 jours après l'éclosion. Une conséquence de ces différences entre les espèces est qu'un délai forcé de l'accouplement (de 3 ou de 10 jours) a moins d'impact sur *A. whitei* et *A. nemoralis* que sur *A. tomentosus*, en ce qui a trait à la longueur de la période de pré-ponte mesurée à partir du moment de l'accouplement. Les femelles accouplées d'*A. nemoralis* et d'*A. whitei* développent des oocytes plus grands que les femelles non accouplées en moins de 2 jours de l'accouplement; le même phénomène prend 4 jours chez *A. tomentosus*. Les embryons sont visibles dans les oeufs de femelles d'*A. nemoralis* et d'*A. whitei* accouplées en 2–3 jours après l'accouplement et en 5 jours après l'accouplement chez *A. tomentosus*. Les oeufs à maturité avec opercule sont visibles respectivement aux jours 3, 4 et 6 après l'accouplement, chez *A. nemoralis*, *A. whitei* et *A. tomentosus*. Chez les femelles non accouplées des trois espèces, 10 jours après l'éclosion, il y a des concentrations significativement plus importantes de lipides assignés aux tissus non reproductifs que chez des femelles de même âge qui se sont accouplées le jour de leur éclosion; cela laisse croire qu'il y a un compromis entre l'allocation des ressources aux oeufs et aux réserves somatiques.

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Introduction

Predatory bugs in the Anthocoridae (Hemiptera) are important natural enemies of soft-bodied arthropods in both agricultural and

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natural habitats throughout the world (Lattin 1999). For many species in this family, information on aspects of basic biology is lacking (Lattin 1999, 2000), including information on mating and reproductive biology. The true bugs (Hemiptera) exhibit a number of interesting reproductive adaptations, such as hemocoelic (traumatic) insemination, anautogenous reproduction, parental care (including male parental care), and mating-induced ovarian maturation. The last adaptation occurs in the Anthocoridae and related taxa (Cimicidae) and is characterized by an inhibition of oocyte maturation in unmated females (Anderson 1962; Davis 1964; Shimizu 1967; Carayon 1970). The anthocorid and cimicid eggs lack a true micropyle, and fertilization takes place in the ovaries before the chorion is deposited and before the egg has moved into the oviduct (Southwood 1956; Cobben 1968). In both the Anthocoridae and the Cimicidae, sperm must move from the site of insemination to the ovaries, either through the hemocoel or through specialized conductive tissues in the female (Carayon 1953; Davis 1956; Hinton 1964). Unmated females in the Anthocoridae and Cimicidae deposit few or no eggs (Mellanby 1939; Lee 1954; Anderson 1962; Davis 1964; Shimizu 1967; Carayon 1970).

Here, we describe the effects of mating on several reproductive characteristics in females of three species of *Anthocoris* (Hemiptera: Anthocoridae) found inhabiting the Pacific Northwest: *A. tomentosus* Péricart, *A. whitei* Reuter, and *A. nemoralis* (Fabr.). We had three objectives. First, we wanted to determine whether mating affects rate of oocyte development in the three species and compare the species with respect to the extent of oocyte development shown by virgin females. Quantitative comparisons of oocyte maturation in mated and unmated females of Cimicidae are available (Davis 1964), but similar studies of Anthocoridae have not been conducted. Second, we wished to determine whether absence of mating leads to higher levels of lipid reserves than those seen in mated individuals. Egg production in insects is known to be costly and may lead to trade-offs in resources allocated to reproduction versus other processes (Legaspi and O'Neil 1994; Ellers 1996). Preliminary observations had suggested that there was a trade-off between somatic fat reserves and oocyte development in *Anthocoris* spp., and that a delay in mating led to visibly large reserves of fat. This hypothesis was addressed quantitatively. Lastly, we wanted to compare the three species in terms of how a forced delay in mating affects duration of the preoviposition period (defined here as the interval between mating and onset of egg laying). As we show in the first part of this paper, the three species differ in the extent to which oocytes develop in unmated females. We hypothesized that the two species in which some oocyte development occurs in virgin females would exhibit a shortening of the preoviposition period associated with a mating delay, relative to the species in which no ovarian maturation is observed in virgins.

Materials and methods

Source of insects

Cultures of *Anthocoris* bugs were begun from field-collected insects. Beating trays were used to collect adults and nymphs of *A. tomentosus* and *A. whitei* from deciduous trees and shrubs growing west of Tieton, Yakima Co., Washington. *Anthocoris nemoralis* was collected from *Acacia longifolia* (Andr.) Willd. (Fabaceae) located near Richmond, Contra Costa Co., California, and from pear orchards located at Hood River, Hood River Co., Oregon. Most assays used first-generation offspring of the field-collected bugs, but the distances we were required to travel while collecting *A. nemoralis* meant that some tests with this species were done using insects that had been in culture for two or three generations.

The three species were reared in the laboratory on pear leaves (*Pyrus communis* L. 'Bartlett'; Rosaceae) infested with pear psylla, *Cacopsylla pyricola* (Förster) (Hemiptera: Psyllidae). Early-instar predator nymphs were reared in groups of two or three individuals in glass petri dishes lined with filter paper. Infested pear leaves were added daily to the dishes. Once the bugs had molted to the fifth instar, they were separated and reared individually in petri dishes. As they molted to the adult stage, date of eclosion and sex of the insects were recorded. These adults were then used in the experiments. Rearing and assays were done in environmental chambers maintained at 22 ± 3 °C with a photoperiod of 16L:8D.

Voucher specimens of all three species have been placed in the M.T. James Entomological Museum at Washington State University, Department of Entomology, Pullman, Washington.

Effects of mating on ovarian development

Growth rates of oocytes were compared among species and between mated and virgin females within species. Newly eclosed (within 24 h) females of *A. tomentosus*, *A. nemoralis*, and *A. whitei* were paired with previously unmated males of 2–5 days of age in petri dishes. Once mating was observed (onset generally within a few minutes of the bugs having been placed in the petri dishes) and copulation had ended, the females were removed from the petri dishes and caged individually on psylla-infested pear seedlings. Seedlings were covered with 135-mL screened cages. A similar number of newly eclosed unmated females were placed individually on pear seedlings.

Females from both mating groups (*i.e.*, mated or unmated; hereafter, mating status) were removed at 2-day intervals and dissected to determine size of oocytes. Samples were removed at 0 (newly eclosed virgins), 2, 4, and 6 days after eclosion for *A. nemoralis* and *A. whitei* and at 0, 2, 4, 6, and 8 days after eclosion for *A. tomentosus*. Females were dissected in saline under a dissecting microscope equipped with an ocular micrometer. Length of the basal oocyte (*i.e.*, the oocyte nearest the oviduct) was measured. In some older unmated females, basal oocytes often showed signs of resorption (oocyte of irregular shape, having granular appearance). Oocytes having these characteristics were not measured. Instead, either the oocyte above the resorbing egg or a basal oocyte from a different ovariole was measured (depending upon which was larger). In mated females, eggs that had developed sufficiently to have moved out of the ovariole and into the oviduct were not measured. Sample sizes for each species were 5–8 females per age class and mating status. Effects of female age and mating status on oocyte length were assessed with a two-way analysis of variance using PROC GLM (SAS Institute Inc. 2001). If the interaction term was significant, oocyte length was compared between mated and virgin females for each age class separately by extracting simple effects contrasts (Winer 1971) in PROC GLM using CONTRAST statements.

We also compared the three species in terms of how rapidly oocytes developed in mated females by monitoring timing of embryo formation and egg cap formation (Cobben 1968). Samples of each species were collected from the mated group at 1- or 2-day intervals. Mated females were collected at 4, 6, and 8 days (*A. tomentosus*) or at 2, 3, and 4 days (*A. nemoralis* and *A. whitei*) after pairing. Each insect was killed by crushing its head and was then dissected using insect pins and a scalpel. The abdomen was gently pulled from the thorax far enough that the ovary suspensory ligaments could be cut loose from their attachments. The basal half of the abdomen was then cut open along a lateral margin. Finally, the abdomen was pulled apart between the 6th and 7th segments, and the terminal segments with ovaries attached were pulled away from the abdomen. This procedure kept incompletely formed eggs from being stretched and distorted. Remaining sternites were teased off to expose the ovary base. Each ovary was then torn off at the lateral oviduct. If a membrane still remained around the ovaries, it was teased open to expose the ovariole strands.

The eggs were dyed following the procedure of Cobben (1968, p. 5). The ovaries were placed in a small beaker of simmering 70% ethanol for 1 min. Shortly after cooling, eggs that had matured enough to have a chorion were pricked with a sharpened insect pin. The material was then transferred to a solution of Grenacher's alcoholic borax carmine for 1 day, moved to acid alcohol (0.2% glacial acetic acid, 35% ethanol) for 30 to 60 min, and placed in lactic acid (85%) for 15 to 30 min. The stained ovary material was then moved to 75% ethanol and immediately examined at 50× under a stereomicroscope. The eggs with the most advanced development were examined. We categorized egg maturation using the scheme of Cobben (1968, p. 133) for *Anthocoris nemorum* (L.): embryo not readily visible (Cobben's stage A); embryo readily visible and with an obvious S shape (Cobben's stages B–D); and egg with an obvious cap (Cobben's stages E–F). Sample sizes were 4–10 females per age class and species. The variability in sample size was due to irregular success in staining the eggs.

Effects of mating on somatic lipid reserves

We tested whether absence of mating in these three *Anthocoris* species resulted in larger quantities of lipids associated with somatic tissues than found in mated females. Newly eclosed females were paired individually with 2- to 5-day-old males in petri dishes. Once a female was observed to have been mated, she was removed from the petri dish and placed in a 135-mL screened cage on a small pear seedling infested with pear psylla. Sample sizes were 10

females per species. The same number of newly eclosed unpaired females was placed in a second set of 30 cages. Each female was removed from the plant when she was 10 days old and stored at –80 °C for 2–4 weeks until she was assayed for lipid content. Storage time varied among females owing to differences among females in date of eclosion.

Females were removed from the freezer, allowed to thaw for 15 min at room temperature, and then weighed individually to the nearest 0.1 mg on a microbalance to obtain whole-body fresh mass. Tissues were assayed for total lipid using a modification of the vanillin reaction (Van Handel 1985). The anthocorids were dissected in a drop of saline on a microscope slide using insect pins. The abdomen was removed from the insect and slit open. Eggs, ovarioles, and adhering connective tissue were lifted from the insect using a pin or a microspatula and discarded. All remaining body parts were placed in a small amount (approximately 0.2 mL) of saline solution in a 1.5-mL microcentrifuge tube. To each of these tubes was added 200 µL of a 2:1 (*v*/*v*) mixture of chloroform:methanol. The tissues were then homogenized using a plastic pestle, and an additional 300 µL of the chloroform:methanol solution was added. The tubes were vortexed to mix the solution and then centrifuged at 14 000 r/min (23 000 *g*) for 5 min at room temperature in a microcentrifuge. The methanol phase was removed and duplicate 100-µL aliquots of the organic phase were added to 16 mm × 150 mm glass test tubes. The extract was dried at 65 °C. Standards consisted of corn oil in chloroform at a concentration of 1 mg/mL and were tested in duplicate using aliquots of 0, 5, 10, 25, 50, 75, and 100 µL. Each standard and test sample received 200 µL of H_2SO_4. The test tubes were heated for 10 min at 90–100 °C. After heating, 5 mL of the vanillin reagent was added to each tube. Color development occurred in 5 to 30 min. Absorbance at 595 nm was recorded.

Lipid quantities were compared between mated and unmated bugs using analysis of covariance (PROC GLM, SAS Institute Inc. 2001), with fresh body mass included as a covariate. The response variable was total lipids (in micrograms), excluding lipids in the ovaries and developing or developed eggs. We first fitted models containing the treatment (*i.e.*, matedness) × covariate interaction to ensure that regression slopes were the same for mated and unmated bugs. In all cases, the interaction term was nonsignificant ($P > 0.2$). We then fitted the standard ANCOVA models, which assume parallel regression lines for mated and unmated bugs. A second analysis was done to support the ANCOVA results by first expressing lipid quantities as ratios (micrograms of lipids per milligram of fresh body mass) and then comparing these ratios between mated and unmated females using *t* tests (PROC TTEST, SAS Institute Inc. 2001).

Effects of forced mating delays on duration of the preoviposition period

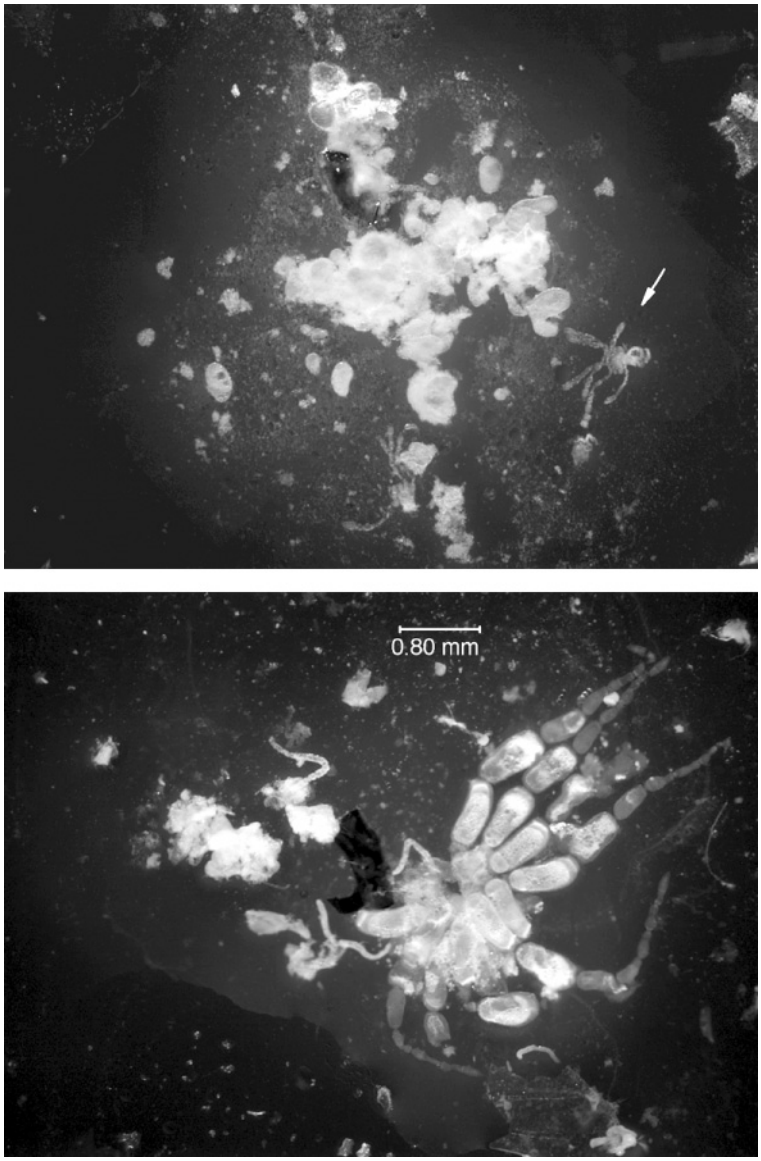
We examined the extent to which a forced mating delay affected duration of the preoviposition period (defined here as the interval between mating and onset of egg laying) in the three species of *Anthocoris* by allowing females to mate either on the day after eclosion or at 10 days after eclosion. Virgin females were collected within 24 h of eclosion and either paired with males immediately or held for 10 days before being paired. All females were kept in screened cages identical to those used in the other experiments and were fed nymphs and eggs of pear psylla. Males and females remained paired for the duration of the experiment. After the insects had been paired, pear seedlings and insects were placed in an environmental chamber under the conditions described earlier. Seedlings were examined daily for the first appearance of eggs. Sample sizes were 10–15 females per treatment and species. The study was then repeated for all three species using a 3-day delay in mating rather than a 10-day delay, with sample sizes of 10–12 females per treatment and species. Duration of the preoviposition period was compared between females that experienced a forced mating delay and those that were mated immediately using two-sample *t* tests (PROC TTEST, SAS Institute Inc. 2001).

Results

Effects of mating on ovarian development

Extent of ovarian development differed noticeably between mated and unmated bugs (example for 10-day-old *A. tomentosus* in Fig. 1). Oocytes in mated females developed more rapidly in *A. whitei* and *A. nemoralis* than in *A. tomentosus* (Fig. 2). Two-way (age × mating status) ANOVA was used to analyze oocyte length, excluding newly eclosed insects (day 0).

Fig. 1. Ovarian development in 10-day-old *Anthocoris tomentosus* females: upper panel, unmated female; lower panel, female mated on the day of eclosion. Magnification is equivalent in the two photographs. The arrow (upper panel) points to ovaries in the unmated female. Whitish material prevalent in the upper panel is fat body.



The tests showed significant age × status effects for *A. tomentosus* ($F_{3,41} = 45.1$, $P < 0.001$) and *A. whitei* ($F_{2,45} = 18.9$, $P < 0.001$). Simple effects contrasts (*i.e.*, comparisons of mated and unmated females for each age separately) showed that oocyte length differed between mated and unmated females by 4 days after eclosion in *A. tomentosus* (Fig. 2; $F_{1,41} = 20.1$, $P < 0.001$) and by 2 days after eclosion in *A. whitei* ($F_{1,45} = 20.5$, $P < 0.001$). For *A. nemoralis*, the age × status interaction was nonsignificant, and a test on the main effect of mating status indicated that oocyte length was greater in mated than in unmated females ($F_{1,31} = 66.4$, $P < 0.001$). We conducted a one-way ANOVA to test whether age affected oocyte length in unmated females, and included in the analyses data for the newly eclosed (day 0) females. Oocytes increased in size with increasing age of the unmated female for both

Fig. 2. Mean (±SE) length of largest oocyte in mated (squares) and unmated (circles) females of different ages for *Anthocoris whitei*, *A. nemoralis*, and *A. tomentosus*. Data at day 0 are for newly eclosed and unmated females. Missing error bars are smaller than the symbols. Arrows indicate when S-shaped embryos (EM) and egg caps (EC) first appeared.

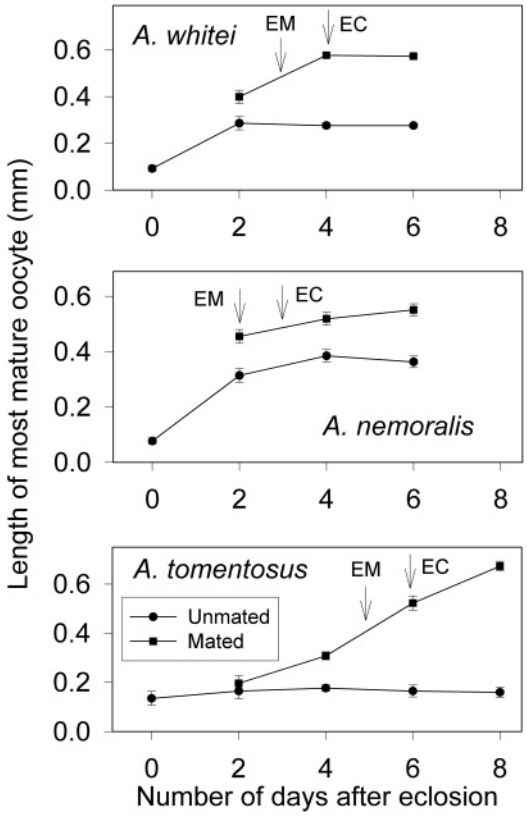
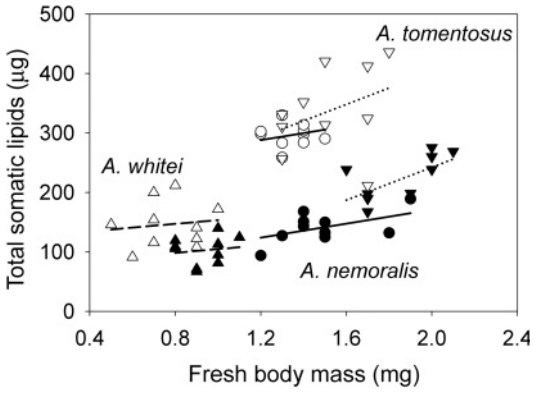


Fig. 3. Total lipids in 10-day-old mated and unmated *Anthocoris whitei* (triangles and dashed lines), *A. nemoralis* (circles and solid lines), and *A. tomentosus* (inverted triangles and dotted lines) from which ovarian tissues had been removed. Open symbols, virgin females; solid symbols, mated females. Lines are regressions from the ANCOVAs.



A. whitei and *A. nemoralis* ($P < 0.001$ for both species), but not for *A. tomentosus* ($F_{4,27} = 1.0$, $P = 0.42$).

The S-shaped embryo (stages B–D of Cobben 1968) first became visible 2, 3, and 5 days after mating in *A. nemoralis*, *A. whitei*, and *A. tomentosus*, respectively (Fig. 2). The egg cap was visible by days 3, 4, and 6 in *A. nemoralis*, *A. whitei*, and *A. tomentosus*, respectively (Fig. 2).

Effects of mating on somatic lipid reserves

Dissections suggested that unmated females had larger amounts of fat reserves than mated females of the same age (Fig. 1). This hypothesis was addressed quantitatively by estimating lipid content in mated and unmated females. Quantity of total lipids remaining after the removal of ovaries was larger in unmated bugs than in mated bugs for all three species (ANCOVA; *A. nemoralis*, $F_{2,17} = 242.6$, $P < 0.001$; *A. tomentosus*, $F_{2,17} = 24.3$, $P < 0.001$; *A. whitei*, $F_{2,17} = 7.8$, $P = 0.013$; Fig. 3). The covariate term was not significant for any of the three species ($P = 0.069$, 0.060, and 0.59 for *A. nemoralis*, *A. tomentosus*, and *A. whitei*, respectively).

If lipid quantities are expressed relative to fresh body mass, virgin females had larger quantities of somatic lipids than mated females in all three species (µg lipids/mg fresh body mass, mean ± SE: *A. nemoralis*: mated, 95.0 ± 4.5; unmated, 221.7 ± 7.0; $t_{18} = 15.2$, $P < 0.001$; *A. tomentosus*: mated, 119.3 ± 5.3; unmated, 223.2 ± 14.0; $t_{18} = 6.9$, $P < 0.001$; *A. whitei*: mated, 111.2 ± 8.7; unmated, 196.4 ± 20.2; $t_{18} = 3.9$, $P < 0.001$).

Effects of forced mating delays on duration of the preoviposition period

Forced delays in mating had no effects on number of days between mating and onset of egg laying in *A. tomentosus* (Table 1). In this species, females began egg laying about 8 days (7.8–8.3 days) after mating, regardless of whether mating delays occurred (Table 1). Conversely, females of *A. whitei* and *A. nemoralis* that experienced delays in mating required significantly less time following mating to begin egg laying than females that were mated on the day of eclosion (Table 1). For example, *A. nemoralis* required a mean of 3.0 days following mating to

Table 1. Mean (±SE) number of days (number of females (*n*) in parentheses) between male–female pairing and deposition of first egg in three species of *Anthocoris* that mated within 24 h of eclosion (no delay) or 3 or 10 days after eclosion (mating delay).

	Preoviposition period (no. of days between mating and onset of egg laying)		
Comparison	No delay	Mating delay	*P**
	A. tomentosus		
No delay vs. 3-day delay	7.9±0.2 (12)	8.1±0.2 (10)	0.55
No delay vs. 10-day delay	7.8±0.2 (11)	8.3±0.2 (12)	0.15
	A. nemoralis		
No delay vs. 3-day delay	4.3±0.2 (9)	3.0±0.2 (11)	<0.001
No delay vs. 10-day delay	4.2±0.2 (15)	3.5±0.2 (12)	0.030
	A. whitei		
No delay vs. 3-day delay	4.5±0.2 (10)	4.1±0.2 (11)	0.20
No delay vs. 10-day delay	5.0±0.3 (10)	3.6±0.3 (11)	0.002

*P value from two-sample t test; compares no delay versus mating delay.

initiate egg laying if mating was delayed for 3 days following eclosion, whereas a mean of 4.3 days was required if females were allowed to mate within 24 h of eclosion (Table 1).

Discussion

Mated females of *A. tomentosus*, *A. nemoralis*, and *A. whitei* differed in rate of oocyte development. Egg maturation indicators, such as the first appearance of the embryo (Fig. 2: EM) or the first appearance of the egg cap (Fig. 2: EC), indicated that females of *A. tomentosus* exhibited slower development (by 2–3 days) than *A. nemoralis* or *A. whitei*. Duration of the preoviposition period in females mated within 24 h of eclosion was 3–4 days longer in *A. tomentosus* than in *A. nemoralis* or *A. whitei* (Table 1). Estimates of the duration of the preoviposition period in *A. tomentosus* and *A. nemoralis* reported here (Table 1) are consistent with results published elsewhere (Horton *et al.* 1998, 2000).

Delays in mating had noticeable effects on ovarian development in all three species. An absence of mating slowed oocyte development and prevented full maturation of eggs (Figs. 1, 2). These effects were eliminated by allowing mating (Table 1). In all three species, a forced delay in mating also led to a delay in the onset of egg laying. The magnitude of effects associated with mating delays differed among species. In *A. tomentosus*, oocyte development was completely inhibited in unmated females (Fig. 2), and a mating delay had no effect on duration of the preoviposition period (defined here as the interval between mating and onset of egg laying) (Table 1). Unmated females of *A. nemoralis* and *A. whitei*, on the other hand, exhibited some oocyte development. Consequently, in these two species, females that experienced a forced delay in mating had significantly shorter preoviposition periods than females that were mated within 24 h of eclosion (Table 1).

Absence of mating, and hence delayed ovarian development, was also associated with larger quantities of somatic lipid reserves compared with quantities found in mated females (Fig. 3), suggesting that there is a trade-off between reproduction and allocation of reserves to other metabolic processes. Egg production has been shown to have costs in terms of lipid reserves in other insects (Ellers 1996; Ziegler and Ibrahim 2001), including other Hemiptera (Legaspi and Legaspi 1998). Trade-offs in resource allocation among life-history processes are common in the Insecta and often occur in response to deteriorating environments (Southwood 1977; Angelo and Slansky 1984; Nylin and Gotthard 1998) or an absence of mates, as shown here. In some predatory insects, deteriorating food supplies prompt decreases in egg production accompanied by increases in lipid reserves (Legaspi and Legaspi 1998; see Anderson 1962 for *Anthocoris*). Less

is known about female responses to shortages of mates, although results reported here and elsewhere suggest that shortages of mates may also prompt reallocation of resources through, for example, resorption of eggs (Davis 1964; Bell and Bohm 1975) or a change in how lipid reserves are allocated between reproduction and somatic reserves (Fig. 3).

Mating may affect any of several reproductive activities in female insects, including onset or rate of ovarian development, length of the preoviposition period, length of the egg-laying period, fecundity, and mating receptivity (Davis 1964, 1965; Engelmann 1970; Eberhard 1996). In this study we have shown that delays in mating for three species of *Anthocoris* lead to delays in oocyte development and cause a prolongation of the preoviposition period. Moreover, unmated females of all three species showed increased levels of somatic lipid reserves, suggesting that ovarian development in mated females occurs at some cost in terms of resources that might otherwise be used elsewhere (Legaspi and Legaspi 1998). It is unclear how these reproductive characteristics affect *Anthocoris* biology in natural conditions, as little is known about mating behavior outside of the laboratory (Horton *et al.* 2000, 2001, 2002). Our results here suggest that females that might experience delays in mating in the field, perhaps under conditions of low density, would experience potentially important consequences in terms of life-history characteristics, including delayed onset of egg laying.

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