

# Sperm competition in the damselfly *Enallagma hageni* Walsh (Odonata: Coenagrionidae): benefits of multiple mating to males and females

Ola M. Fincke\*

Program in Evolutionary Ecology and Behavior, Department of Zoology, The University of Iowa, Iowa City, Iowa 52242, USA

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**Summary.** Sperm competition was investigated in the non-territorial damselfly, *Enallagma hageni*. Using irradiated (sterile) male techniques, the last male to mate was found to fertilize up to 95% ( $\bar{x}=80\%$ ) of the eggs of the first clutch laid after mating. Dissection of females collected before, during, and after copula showed that a male removes a maximum of 87% of the sperm by volume of a previous mate. These data verify an earlier estimate of lifetime reproductive success in this species which was based on mating success, and suggest that indirect dissection methods offer minimum estimates of sperm precedence. Male *E. hageni* have ample opportunity to benefit from sperm precedence, since at least 10% of the receptive females encountered had already mated once that day, but still contained complete or partial clutches of eggs. Female *E. hageni* benefit directly from high sperm precedence because it allows them to “exchange” matings for guarding service by males during oviposition bouts under water.

## Introduction

Because female insects retain sperm in storage organs, and may mate repeatedly before ovipositing, the opportunity exists for post-copulatory competition among males by displacing or removing sperm of a previous mate (Parker 1970). Sperm competition may in turn select for behaviors by which males can protect their sperm investment, either mechanically (Labine 1966; Craig 1967), chemically (Riemann et al. 1967), or behaviorally by prolonged copulation (Mason 1980; Sillén-Tullberg 1981), or postcopulatory guarding (Waage 1979a; Loher 1981; Fincke 1982; Johnson 1982).

While many laboratory studies have demonstrated that the last male to mate fertilizes most of a female's clutch, considerable sperm mixing has also been found in some insects (see review in Boorman and Parker 1976; Bartlett et al. 1968; Woodhead 1983). The effect of a high degree of sperm precedence on male behavior in a few natural populations has been demonstrated (Parker 1978; Waage 1979a). More often however, direct evidence of sperm competition in field studies is lacking and last male precedence is inferred from behavioral data. Sperm competition has been predicted for example, in field studies estimating lifetime reproductive success in insects (Fincke 1982; McCauley 1983), and those assessing the results of sexual selection (e.g. Jacobs 1955; Mason 1980; Thornhill 1980), or the success of alternative male mating tactics (Campanella and Wolf 1974; Alcock et al. 1979; Mangan 1979). In none of these studies was the degree of sperm competition known.

While sperm competition obviously affects male fitness, the possibility for such competition depends upon multiple mating by females. Theoretical benefits of multiple matings for females have been addressed (Parker 1970; Boorman and Parker 1976; Walker 1980; Waage 1983), but empirical evidence of selective advantages of repeated matings for females is rare (Waage 1979a; McLain 1981), except where females gain nutrients from a male's spermatophore or accessory gland secretions (Pease 1968; Boogs 1981). Knowing the degree and mechanism of sperm competition, and its effects on both males and females is essential for an understanding of the evolution of reproductive behavior in insects.

Within the order Odonata, the mechanism of sperm competition is known in several species. Waage (1979b, 1983) has shown by dissection, that many species of zygoptera and libellulids use the penis to completely or partially remove sperm of

\* Present address: Smithsonian Tropical Research Institute, APO 34002, Miami, Florida, USA

a previous mate from the female's storage organs. In a few odonates, males displace sperm by packing it farther into the female's storage organs rather than by removal (Miller 1981; Waage 1982).

Dissection methods do not measure the consequences of sperm removal in terms of the proportion of eggs fertilized by a second mate. Measurement of the fertilization success of a second mate requires labeling sperm either by irradiation, or with genetic markers. Neither technique has previously been used on odonates.

I report here the results of my studies of sperm competition in *Enallagma hageni*. I used irradiated male techniques to determine the degree of sperm precedence, and dissection of in copula pairs to determine whether sperm is displaced by removal or packing. I then examined the consequences of sperm displacement for females as well as for males in light of the mating behavior in a natural population.

## Materials and methods

**Reproductive behavior.** Females become receptive when they have matured a clutch of eggs ( $\bar{x}$  interclutch interval =  $5.2 \pm 0.6$  days). A male initiates a copulation attempt by seizing a female in tandem. Male damselflies cannot force females to mate because a female must raise her abdomen to the male's second abdominal segment to engage the genitalia. Unreceptive females grasped by males resist them by clinging to their perch, and are usually released within 1–10 min.

In this paper, copulation refers to the time during which male and female genitalia are in contact. Insemination refers to the stage of copulation during which the male transfers sperm to the female from his temporary sperm storage sac, the penis vesicle (located just behind the penis). After copulation, which averages 22 min, the pair remains in tandem ( $\bar{x}$  = 58 min) until the female submerges to oviposit at the base of aquatic plant stems. While a female is submerged, her mate remains on the perch above her, and usually seizes her when she resurfaces. A female will mate with up to four different males in a single day, provided she has not yet laid her entire clutch. Males do not guard females unless they have mated with them at least once (Fincke 1983).

In the summer of 1982, teneral (virgin) females were collected and raised to adulthood (4–7 days) in a field insectary (3 m  $\times$  1.2 m  $\times$  2 m) at the University of Michigan Biological Station near Pellston, Mich. Normal feeding and mating behavior occurred in the screened enclosure, where individuals could be kept alive for up to 3 weeks. All matings described below were conducted in the insectary.

**Irradiation methods.** For the irradiation experiments, males were field collected in early afternoon, individually marked by writing a number on the wing with a felt-tip pen, placed in small cages in a cooler (kept at 15 °C), and transported to the radiation lab of a local hospital 30 min away. Five to six males were put in a plastic petri dish and exposed to 22.3 min of X-ray radiation (200 KVp, HVL = 0.25 mm Cu) at a distance of 25.5 cm from the source, resulting in an exposure of 14.7 Kilo-Roentgens. A total of 30 males was irradiated. Control males were treated identically but were not irradiated. An irra-

diated male was dissected and its testes squashed and examined under a light microscope to determine that the sperm were still active. Total time from collection in the field to release in the insectary was 3 h and 20 min.

Irradiated and non-irradiated males were allowed to mate with mature, virgin females reared in the insectary. Five control females were mated once and the remainder allowed to mate a second time with either an irradiated or nonirradiated male. All matings occurred within 4–48 h of the X-ray treatment. Copulation durations were measured for most of the matings. Females were then isolated in small cages equipped with moistened filter paper. From a total of 43 matings, 14 females lived long enough to deposit eggs within 48 h. Eggs laid in the filter paper were transferred to small plastic rearing vials, which were floated in tubs of water kept at room temperature (20–22 °C). Holes in the vials covered with fine mesh bolting cloth allowed water flow through them.

After 12 days, the eggs were counted and scored as developing or sterile. In normal development, dark eye spots are clearly visible in the embryos by 12 days. An egg was scored as sterile if no such eye spot was found. Total time to hatching under lab conditions was 14–18 days. I used the eye spot stage rather than hatching as a criterion for determining percent of non-sterile eggs to minimize the effect of lab rearing conditions.

**Dissection methods.** To determine the extent to which sperm is mechanically displaced during mating, I dissected females collected either before, during, or after copulation. Thirty-seven females found in tandem with males (and presumably inseminated by them) were field collected in late afternoon. Thirteen of these females were frozen and later dissected (mated controls); the others were allowed to mate with field collected males. I interrupted the copulation of 12 pairs by cutting the male's abdomen just below the thorax, at varying times after copulation was initiated. The female and attached male abdomen were frozen and dissected later (interrupted copula). The remaining twelve pairs were allowed to complete copulation, and the females were then likewise frozen and dissected (post-copula females). To determine how much sperm females carry after fertilization of a clutch, an additional 11 females were collected after oviposition bouts of 25 min or longer, and later dissected.

The two sperm storage organs in *E. hageni* consist of a large bursa copulatrix at the vagina/oviduct junction, to which is attached a single spermatheca. These two female organs and the male's penis vesicle were removed by dissection and soaked for 2–3 min in a 1:1 solution of 70% ethanol and acetic acid, which facilitated the removal of the surrounding tissue (see Waage 1982). The length, width, and depth of each storage organ was measured at 45 $\times$  magnification using an ocular micrometer of a dissecting microscope. Volumes of the storage organs (calculated by multiplying the three measurements and assuming a rectangular-shaped organ) of females interrupted in copula were compared with those of mated controls, postcopula, and postoviposition females. All means are reported with their standard errors.

## Results

### *Irradiation experiment*

The results of the sperm precedence experiment are presented in Table 1. Development occurred in 92.2% of the eggs from control females mated to normal males. In contrast, only 4 eggs (4.5%)

from control females mated to irradiated males developed to the "eye spot" stage. Because 7.8% of the control clutches were found to be sterile, I conclude that 14.7 KR of radiation resulted in 92% (87.7–95.5%) sterilization. After 15 males were exposed to 20 KR of X-ray radiation, none of them later mated and all were dead within 24 h. Exposure to intermediate levels were not given due to time constraints and lack of teneral (i.e. known virgin) females later in the season.

Comparison of the eggs from doubly mated females shows that the second male fertilizes most of the subsequent clutch. Irradiated sperm were not exactly comparable to nonirradiated sperm in the degree of precedence (Table 1). Displacement of normal sperm by irradiated sperm ( $\bar{x}=68.6\%$ ,  $SD=11.7$ ) was significantly less than the displacement of irradiated sperm by non-irradiated sperm

( $\bar{x}=90.4\%$ ,  $SD=5.4$ ), although Table 1 shows that some irradiated sperm did equally well. I therefore estimate that average sperm precedence by a second male varies from a mean of 79.5% to as high as 95%.

#### Dissection data

All but one of the 25 (96%) control and postcopulatory female samples had full to moderate sperm volumes in the bursae (Table 2). Even after fertilizing a clutch of eggs, most females contained nearly full sperm loads. In contrast, 54% of the interrupted females had empty or near empty bursae. The average displacement from the bursa was found to be 82.9%, while the maximum bursa volume difference was 86.7%. To calculate the maximum volume of sperm removed from the bursa, I averaged the bursa volume of the interrupted copula females with empty or near empty bursae ( $n=6$ ), and divided by the average bursa volume of control and postcopula females.

The presence of sperm in the sperm vesicle varied inversely with the bursa volume in all but one case, suggesting that empty bursae were those from which sperm had been scooped but not yet inseminated by the current male. The data suggest sperm is removed from the bursa during the first half of copula, and new sperm added during the last half of copula.

Fullness of the spermatheca varied independently from fullness of the bursa. Roughly 1/3 of the mated control females had empty spermathecae, and 2 of the 12 postcopulatory females did. Only one of the 6 interrupted females that had empty bursae also had no sperm in the spermathecae. It is thus unclear whether sperm moves from the spermatheca into the bursa as sperm from the latter organ are removed. Using the same six females as in the maximum displacement calculations, the average difference in total bursa plus spermathecal volumes between interrupted females

**Table 1.** Egg development following matings with normal and irradiated males. Data reported are the % of a clutch fertilized by the last male. *N* normal males; *R* irradiated males

Mating type	Number of eggs	% developing eggs	% eggs fertilized by second male
<i>Controls</i>			
N	75	88.0	
N	77	89.6	
N	226	94.6	
N	164	96.0	
R	33	3.0	
R	50	6.0	
<i>Experimentals</i>			
N-R	28	7.1	92.9
N-R	113	15.9	84.1
N-R	27	46.9	53.1
N-R	283	55.6	44.4
R-N	388	95.0	95.0
R-N	61	91.8	91.8
R-N	159	84.5	84.5

**Table 2.** Volumes of sperm in female sperm storage organs at various stages in the mating cycle. Data for each storage organ are mean volume and number of specimens in each volume class. Volume classes are: bursa volume (–) 0.01–0.02 mm<sup>3</sup>, (+) 0.04–0.08 mm<sup>3</sup>, (++) 0.12–0.17 mm<sup>3</sup>; spermatheca volumes: (–) 0.001–0.002 mm<sup>3</sup>, (+) 0.004–0.008 mm<sup>3</sup>, (++) 0.01–0.02 mm<sup>3</sup>

Female type	<i>n</i>	Bursa copulatrix			Spermatheca					
		Mean volume (mm <sup>3</sup> )	–	+	++	Mean volume (mm <sup>3</sup> )	–	+	++	?
Mated control	13	0.10±0.01	1	3	9	0.008±0.003	4	6	3	
Interrupted copula	12	0.05±0.02	6	4	2	0.016±0.003	1	4	6	1
Postcopula	12	0.10±0.01	0	4	8	0.012±0.003	2	5	3	
Postoviposition	11	0.06±0.01	2	5	4	0.008±0.003	3	4	3	1

and the combined average of control and postcopula females was 74%.

## Discussion

Males of *E. hageni* were found to remove up to 87% of the sperm by volume from previous matings, enabling the last mate to fertilize from 80–100% of a female's immediate clutch. These results verify earlier estimates of lifetime reproductive success for this species, which were based on the number of matings by males in tandem with ovipositing females, on the assumption that the last male to mate with a female fertilized all of her eggs (Fincke 1982, 1983). The results are also important because they confirm past studies estimating sperm precedence based only on dissection techniques (e.g. Waage 1979b, 1983). This suggests that the latter method may be used to obtain a minimum estimate of sperm precedence when irradiation methods are difficult to apply (as they are for many odonates). In this study, actual precedence, measured by the number of eggs fertilized by a second mate, was actually higher than the measurement of sperm displaced by volume. Thus estimates of sperm displacement tend to underestimate sperm precedence.

The estimate of sperm precedence of 80% is a minimum one, since irradiated sperm were poorer competitors than nonirradiated sperm, confirming the results of Riemann and Thorson (1974). The degree of infertility (8%) of the controls mated to non-irradiated males nearly balances the bias in the estimate due to incomplete sterilization (4.5%). If the data are adjusted for both factors, average displacement remains close to 80%. A maximum estimate of displacement would be close to 95–100%, since in at least two cases, the percent of eggs fertilized by a second irradiated male equaled that of non-irradiated controls.

It may be that higher exposures (e.g. 18 KR) would completely block development and yet not affect male mating ability. However, greater exposure may magnify the problem of decreased competitive ability of sperm (Riemann and Thorson 1974). In *E. hageni*, an exposure to 14.7 KR appeared to decrease competitive ability of sperm by 22% relative to normal sperm. The effect of radiation appears to depend in part on the species (in addition to the size of the insect). For example, *Ischnura verticalis* males treated with 20 KR were capable of mating normally for 10 days after treatment, while identical treatment to *E. hageni*, a spe-

cies only slightly larger, resulted in death within 24 h (personal observation).

Since sperm volumes of interrupted females decreased relative to those of mated control females, the mechanism of sperm competition appears to be sperm removal rather than repositioning or packing (assuming that the density of the sperm mass did not change). The estimated 74% removal of sperm by volume is an underestimate, since a maximum estimate requires that pairs be interrupted just after maximum removal but before any new sperm is introduced by the second mate. My data on the timing of removal support similar findings by Miller and Miller (1981). From detailed observations of copulating pairs of *Enallagma cyathigerum*, they concluded that sperm transfer occurred during the last 200–250 s of copula which is characterized by the absence of abdominal "flexing" seen earlier during copulation (and which is also characteristic of *E. hageni*). They found clumps of sperm on the penis head pulled out of the vagina of females during the early phase of copulation and concluded that sperm is pulled out rather than packed deeper into the bursa. They also showed that the anatomy of the *Enallagma cyathigerum* penis does not allow males to penetrate the spermatheca. The morphology of male genitalia in these two species of *Enallagma* is nearly identical (personal observation).

In *E. hageni*, it appears that a second mate cannot remove all of the sperm from a previous mating. Therefore sperm may eventually mix in the spermatheca and bursa and share in fertilizing eggs of later ovipositions. Because of the poor survivorship of females in oviposition cages, I could not directly estimate the effects of possible sperm mixing in the spermatheca in future ovipositions. However, *E. hageni* males must rarely if ever realize any fertilizations resulting from sperm mixing since females mate before oviposition on any given day, and as in most insects, eggs are fertilized just before they are laid. Of hundreds of ovipositions I have watched in marked populations during 1980–1982, I never saw a female oviposit for the first time on a particular day without first being in tandem with a male (who presumably had inseminated her).

*E. hageni* males have ample opportunity to use their sperm displacement ability. Of 266 tandem pairs followed, 65 (24.4%) involved females who had already mated and oviposited with another male earlier that day. Twenty-six (40%) of these females mated and oviposited again that day. Only once did the male of a second tandem not mate with the female before she oviposited.

Results presented here on direct measurement of sperm displacement support my earlier prediction (Fincke 1982), based on the degree to which males exhibit both contact (in tandem) and non-contact guarding of mates after mating, that *E. hageni* males should be able to displace most of the sperm of a previous mate. At high male density, males do not readily release females for submerged oviposition, and tandem oviposition usually precedes submerged oviposition. During submerged oviposition, mates actively defend perch sites (non-contact guarding), grabbing females when they resurface (which they may do before ovipositing a full clutch) (Fincke 1983). At a small pond, males were successful in recovering their original mate after an oviposition bout in 107 of the 171 (63%) cases when the female resurfaced and was grabbed by a male (Fincke 1983).

Since the opportunity for sperm competition depends on females mating more than once, one should expect females to benefit from multiple matings. This is particularly true in odonate species like *E. hageni*, in which females cannot be forced to mate. Multiple matings for *E. hageni* do carry a cost. Copulation is time consuming and second matings usually occur around the water, where females are exposed to risk of predation by frogs and increased harassment by unmated males (Fincke 1982). Sperm replenishment is not a benefit for multiple mating within a clutch, though it may be an advantage in mating again between clutches. Females were shown to carry full sperm loads even after fertilizing a full clutch of eggs. It is unlikely that *E. hageni* females gain nutrients from repeated matings as do some insects (Boggs 1981), since most sperm of previous mates is removed during copulation.

I propose that *E. hageni* females gain from guarding services provided by males, and "exchange" matings for noncontact guarding protection by males. In 1982 at a study site on a small lake, I found that 17% of 284 submerged females floated on the water surface after an oviposition bout (Fincke 1983). Sixty percent of these females were grabbed by a male (of which slightly more than half were mates). Of those not grabbed by a male, 25% died by drowning or predation, and an additional 18% floated until they were lost from view. Thus, by trading matings for guarding protection by males, females increase the probability of laying a complete clutch, and of surviving to mature additional clutches. Selection should act upon female physiology and morphology to facilitate sperm displacement by males, since by increasing his investment in her clutch, a male should

be less likely to abandon a mate until she has finished laying a complete clutch. Waage (1979a) has found a similar benefit for multiple matings in a territorial damselfly, in which females "exchange" matings for access to oviposition sites. Continued work that relates morphology to behavior in natural populations should increase our understanding of the relative control both sexes have in the evolution of sperm competition.

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## References

- Alcock J, Jones CE, Buchmann SL (1979) Male mating strategies in the bee *Centris pallida* Fox (Anthophoridae:Hymenoptera). *Am Nat* 111:145-155
- Bartlett AC, Mattix EB, Wilson NM (1968) Multiple matings and use of sperm in the bollweevil, *Anthonomus grandis*. *Ann Entomol Soc Am* 61:1148-1155
- Boggs CL (1981) Selection pressures affecting male nutrient investment at mating in Heliconiine butterflies. *Evolution* 35:931-940
- Boorman E, Parker GA (1976) Sperm (ejaculate) competition in *Drosophila melanogaster*, and the reproductive value of females to males in relation to female age and mating status. *Ecol Entomol* 1:145-155
- Campanella PJ, Wolf LL (1974) Temporal leks as a mating system in a temperate zone dragonfly (Odonata:Anisoptera). I. *Platthemis lydia*. *Behaviour* 51:49-87
- Craig GB (1967) Mosquitoes: female monogamy induced by male accessory gland substance. *Science* 156:1499-1501
- Fincke OM (1982) Lifetime mating success in a natural population of the damselfly *Enallagma hageni* (Walsh) (Odonata:Coenagrionidae). *Behav Ecol Sociobiol* 10:293-302
- Fincke OM (1983) Lifetime mating patterns and reproductive success in the damselfly, *Enallagma hageni* (Walsh) (Odonata:Coenagrionidae). PhD thesis, University of Iowa
- Jacobs ME (1955) Studies on territorialism and sexual selection in dragonflies. *Ecology* 36:566-586
- Johnson LK (1982) Sexual selection in a brentid weevil. *Evolution* 36:251-262
- Labine PA (1966) Population biology of the butterfly *Euphydryas editha*: IV. Sperm precedence - a preliminary report. *Evolution* 20:580-586
- Lohrer W (1981) The effect of mating on female sexual behavior of *Teleogryllus commodus* Walker. *Behav Ecol Sociobiol* 9:219-225
- Mangan RL (1979) Reproductive behavior of the cactus fly, *Odontoloxozus longicornis*, male territoriality and female guarding as adaptive strategies. *Behav Ecol Sociobiol* 4:265-278
- Mason L (1980) Sexual selection and the evolution of pairbonding in soldier beetles. *Evolution* 34:174-180
- McCauley DE (1983) An estimate of the relative opportunities for natural and sexual selection in a population of milkweed beetles. *Evolution* (in press)

- McLain DK (1981) Interspecific interference competition and mate choice in the soldier beetle, *Chauliognathus pennsylvanicus*. Behav Ecol Sociobiol 9:65–66
- Miller PL (1981) Functional morphology of the penis of *Celithemis eponina* Drury (Anisoptera: Libellulidae). Odonatologica 10:259–354
- Miller PL, Miller CA (1981) Field observations on copulatory behavior in zygoptera with an examination of the structure and activity of the male genitalia. Odonatologica 10:201–218
- Parker GA (1970) Sperm competition and its evolutionary consequences in the insects. Biol Rev 40:525–567
- Parker GA (1978) The reproductive behavior and the nature of sexual selection in *Scatophaga stercoraria* L.: V. The female's behaviour at the oviposition site. Behaviour 37:140–168
- Pease RW (1968) The evolutionary and biological significance of multiple pairing in the Lepidoptera. J Lepid Soc 22:197–209
- Riemann JG, Thorson BJ (1974) Viability and use of sperm after irradiation of the large milkweed bug. Ann Entomol Soc Am 67:871–876
- Riemann JG, Moen DJ, Thorson, BJ (1967) Female monogamy and its control in houseflies. J Insect Physiol 13:407–418
- Sillén-Tullberg B (1981) Prolonged copulation: A male post-copulatory strategy in a promiscuous species *Lygaeus equestris* (Heteroptera: Lygaeidae). Behav Ecol Sociobiol 9:283–289
- Thornhill R (1980) Mate choice in *Hylobittacus apicalis* (Insecta: Mecoptera) and its relation to some models of female choice. Evolution 34:519–538
- Waage JK (1979a) Adaptive significance of postcopulatory guarding of mates and nonmates by male *Calopteryx maculata* (Odonata). Behav Ecol Sociobiol 6:147–154
- Waage JK (1979b) Dual function of the damselfly penis: Sperm removal and transfer. Science 203:916–918
- Waage JK (1982) Sperm displacement by male *Lestes vigilax* Hagen (Zygoptera: Lestidae). Odonatologica 11:201–209
- Waage JK (1983) Sperm competition and the evolution of odonate mating systems. In: Smith RL (ed) Sperm competition and the evolution of animal mating systems. Academic Press, New York, pp 251–290
- Walker WF (1980) Sperm utilization strategies in nonsocial insects. Am Nat 115:780–799
- Woodhead A (1983) Interaction of gamete competition and development on components of male fitness in the cockroach *Diploptera punctata* (Eschscholtz). PhD thesis, University of Iowa