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Repeated Copulation and Sperm Precedence: Paternity Assurance for a Male Brooding Water Bug

Abstract. *Male giant water bugs (*Abedus herberti *Hidalgo) brood eggs attached to their backs by their mates. Brooders risk being "cuckolded" because females store sperm from previous matings. Males always copulate with females prior to receiving their eggs and mate repeatedly during oviposition. Experiments with a genetic marker reveal almost complete sperm precedence for the last male to mate with a female. The male's behavior therefore assures his paternity of the eggs he broods.*

A male should only care for, and take risk in defense of, young that possess his genes. Failure to assure paternity prior to investing in young may put a male at a selective disadvantage in competition with more reproductively selfish individuals (*1*). Observational and experimental studies on birds imply support for this thesis; however, few quantitative data are available to evaluate the efficacy of presumptive "anticuckoldry" adaptations (*2*).

With a few exceptions (*3*), male insects contribute nothing to reproduction beyond sperm. Among the exceptions are males of the giant water bug subfamily Belostomatinae, which invest time and energy brooding eggs attached to their backs by conspecific females (*4, 5*). *Abedus herberti* Hidalgo males brood by aerating eggs and assisting nymphs during eclosion (*5*). In addition, encumbered males do not feed while nymphs are hatching from their backs (*5*). This paternal behavior involves added risks, reduces predatory efficiency, and precludes additional mating for brooding males (*5, 6*). Male brooding, however, is essential for embryonic development under natural conditions (*4, 5*).

Ridley points out that the evolution of exclusive male nurture is correlated with external fertilization, presumably for reasons of paternity assurance (*3*). This is not the case for giant water bugs. Most female insects, including giant water bugs, store sperm in organs (spermathecae) and use it to fertilize eggs laid over an extended period. *Abedus herberti* is long-lived (> 1 year), and an individual female of this species may mate with several males (theoretically as many as 12) in its lifetime (*6*). Thus, it is entirely possible for males to receive and brood eggs from previously mated females. Given this risk, selection should have favored mechanisms that would assure the brooding male's genetic contribution to eggs he carries. Indeed, a high degree of paternity assurance would seem requisite to the evolution and elaboration of male brooding patterns. Given sperm storage by females, paternity assurance mechanisms in this case would necessarily involve competition between or among ejaculates from two or more males.

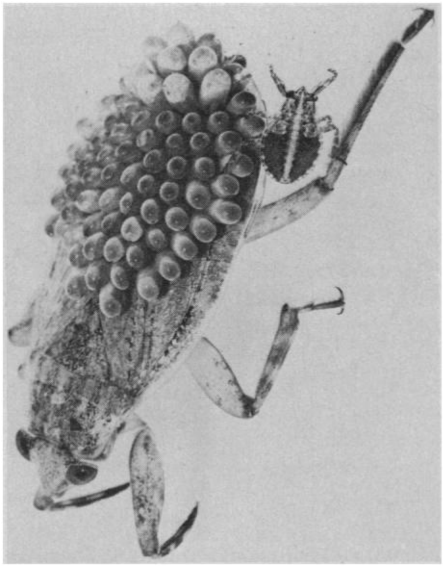

Fig. 1. Wild type (++) vasectomized male with eggs. Eleven swollen eggs on posterior fertilized by a homozygous striped male; not the brooder. Striped nymph recently hatched from one egg.

Such mechanisms seem to occur in the mating behavior of *A. herberti* (*5, 6*). Multiple bouts of copulation always preceded oviposition at male insistence, and copulation and oviposition were cyclical and male-controlled events. Males allowed remarkably consistent time periods for oviposition which were abruptly terminated by repeated coupling. Three eggs were the maximum number laid between bouts of copulation. In the extreme case, a pair coupled over 100 times in 36 hours during transfer of 144 eggs (*6*).

Sperm competition studies have been conducted on a variety of insect species representing several orders (*7, 8*). Of those studied, more than 75 percent exhibited sperm displacement; that is, sperm from the last male to mate predominated in fertilization of subsequently laid eggs. Although no sperm competition studies have been conducted on any Heteroptera, I predicted that sperm displacement or precedence should occur in *A. herberti* because it would provide a male with at least some degree of paternity assurance.

Several lines of evidence suggest that female *A. herberti* retain viable sperm from previous matings. Six of ten gravid females from a natural population near Phoenix, Arizona, had motile sperm in their spermathecae (*6*). Fifteen virgin *A. herberti* females were mated and allowed to deposit their entire complement of eggs on the backs of their respective mates. These females were killed and dissected in randomly selected groups of three at 30, 60, 90, and 120 days after mating. Two died between 120 and 150 days, but the remaining individual was killed and examined 150 days after mating. All 13 spermathecae contained motile sperm. Therefore, a male that receives eggs from a female previously mated up to 5 months before might risk having eggs fertilized by the female's previous mate or mates.

One can only infer risk from the presence of motile sperm in the spermathecae of nonvirgin females. Indisputable evidence would exist if eggs hatched from the backs of sterilized males paired with nonvirgin females. I vasectomized (*9*) a wild-type (++) male and paired him with a ++ female previously mated (30

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days) to a normal male homozygous for a dominant genetic marker, stripe (*10*). The vasectomized male received 75 eggs from this female; however, only 11 of these were fertile as evidenced by their increase in size (*11, 12*). All 11 nymphs that hatched were of the striped phenotype (Fig. 1), hence the vasectomized male had been "cuckolded." This demonstrated a risk to males that receive eggs from nonvirgin females.

To evaluate the efficiency of male mating patterns in minimizing this risk, I conducted sperm competition experiments with virgin ++ females and striped and ++ males. Each of 25 ++ females was mated alternately with a ++ male and a striped male. The order of presentation of males was chosen at random as a control for possible differential competitiveness of their sperm (*7*). Intervals between the first and second competitive matings ranged from 22 to 89 days. Females were permitted to lay all of their eggs on each male's back. I counted the phenotypes of nymphs that hatched from the back of the second males and calculated the proportion attributable to fertilization by the second male. The result (Table 1) indicates precedence by sperm from the second male. Of the 25 second males, 20 succeeded in fertilizing 100 percent of the viable eggs that they received. Excluding replicate 5 (*13*), the average percentage of eggs received and fertilized by all second males was 99.67, the highest level of sperm precedence ever reported for an insect.

Several bouts of copulation with a female prior to accepting her eggs might provide sufficient sperm to assure fertility of all eggs received by a male (*9*). Why then should male water bugs require females to copulate for every few eggs received? This system is expensive for males in terms of sperm as well as in terms of time and added risks for both sexes. It has a measurable cost for females in that they occasionally drop eggs when oviposition is interrupted by male demands for additional coupling (*6*). Male mating patterns may have evolved under intrasexual selection if cyclical polyandry were a constant threat to (and opportunity for) male paternity. I have staged both polygynous and polyandrous mating in the laboratory (*6*) and have some indirect evidence that polygamy occurs under natural conditions (*7*).

I arranged two short-term sperm competition experiments to determine whether cyclical copulation could assure paternity under conditions of multiple maternity and female infidelity. The first experiment consisted of eight replicates of the following procedure. A virgin ++ female was paired with a male of one genotype, was allowed to lay five eggs on his back, and was paired immediately with a second male of the other genotype and allowed to lay the remainder of her eggs. As before, the phenotypes of nymphs that hatched from the second male were tabulated. All eight second males in this experiment succeeded in fertilizing all of the viable eggs (N = 584) they received.

The final experiment consisted of pairing a ++ female alternately with two males of different genotypes (++ and striped). The female was permitted to lay three eggs on the back of one male; she was then removed immediately to the container of the other male. When three eggs had been laid on the second male's back, the female was returned to the original mate. This alternation of mates was repeated until all of the female's eggs had been laid on the backs of two males. Three replicates of the experiment were conducted, and the phenotypes of nymphs that hatched from all six males were recorded. These intensively competitive matings produced only 5 of 250 nymphs attributable to males other than those that had brooded their eggs. This result demonstrated that alternating bouts of copulation and oviposition protect males when polygamy is a threat or an opportunity; but the question remained as to why males should interrupt female oviposition to copulate when no other male is in the vicinity?

Possibly, the present ejaculate displaces alien stored sperm to the blind

Table 1. Results of long-term sperm competition experiments on *Abedus herberti*. Females allowed to mate with and deposit all eggs on first male's back, then after variable intervening time, remated and allowed to deposit a second clutch on second male's back.

Experiment	Phenotype (genotype) of first male	Days between competitive matings 1 and 2	Phenotype (genotype) of second male	Eggs dropped during second mating	Outcome of second mating			Proportion of nymphs attributable to second male
					Striped nymphs	Wild type nymphs	Failed to hatch	
1	Str(SS)	22	Wt(++)	2	2	75	4	0.974
2	Wt(++)	22	Str(SS)	1	100	0	1	1.000
3	Str(SS)	22	Wt(++)	0	0	87	0	1.000
4	Wt(++)	22	Str(SS)	4	86	0	2	1.000
5*	Str(SS)	30	Wt(++)	1	15	6	78	0.286
6	Str(SS)	30	Wt(++)	6	3	108	1	0.973
7	Wt(++)	31	Str(SS)	16	111	0	0	1.000
8	Wt(++)	32	Str(SS)	2	53	0	0	1.000
9	Str(SS)	32	Wt(++)	0	0	73	0	1.000
10	Wt(++)	32	Str(SS)	3	125	0	3	1.000
11	Str(SS)	32	Wt(++)	0	0	92	3	1.000
12	Wt(++)	32	Str(SS)	0	89	0	7	1.000
13	Str(SS)	32	Wt(++)	5	0	63	4	1.000
14	Str(SS)	34	Wt(++)	1	0	58	1	1.000
15	Wt(++)	34	Str(SS)	2	118	0	0	1.000
16	Wt(++)	34	Str(SS)	2	101	0	4	1.000
17	Wt(++)	34	Str(SS)	1	99	0	0	1.000
18	Wt(++)	34	Str(SS)	0	72	0	13	1.000
19	Str(SS)	36	Wt(++)	1	0	79	0	1.000
20	Str(SS)	36	Wt(++)	0	1	73	0	0.987
21	Str(SS)	37	Wt(++)	3	0	93	2	1.000
22	Str(SS)	37	Wt(++)	2	1	72	3	0.986
23	Str(SS)	41	Wt(++)	5	0	75	11	1.000
24	Wt(++)	81	Str(SS)	3	88	0	18	1.000
25	Wt(++)	89	Str(SS)	1	122	0	0	1.000

*Second male died before eggs hatched; eggs artificially brooded, 21 of 99 hatched.

end of the spermatheca. If so, the displaced sperm would remain a threat to the current male. Cyclical copulation may function to repeatedly displace previously stored sperm to the distal end of the spermatheca, thus neutralizing the recurrent threat. The actual mechanism of sperm precedence has been discovered for only one species of insect (*14*). Whether this problem can be resolved for *A. herberti* (*15*) remains to be determined.

In *Scatophaga stercoraria* (L), the yellow dungfly, the last male to mate was responsible for 80 percent of the offspring subsequently produced until another mating occurred. Parker (*16*) determined that males of *S. stercoraria* could probably displace fully all previous sperm by increasing the duration of copulation with each female, but for some reason, dungfly males do not do this. Parker developed a model which revealed that the amount of displacement in *S. stercoraria* should stabilize at the optimum, yielding the maximum overall fertilization rate for males, but not the maximum possible egg gain to a male from a given mating (*8*, *16*).

Abedus herberti males, in constrast, invest in matings and appear to have virtual certainty of paternity. This turns Parker's question around. Why should the water bug paternity assurance system be so nearly perfect? Male water bugs lack the opportunity to optimize their overall fertilization rate because egg-covered males are rejected as mates by females (*6*). Because of this limitation on the absolute number of eggs a male water bug is allowed to fertilize, individual males have apparently been under intense selection to maximize egg gain from each mating. Even the smallest fault in paternity assurance by male brooders would provide an opportunity for cheaters (males that mate repeatedly, but deline to brood eggs); and the frequency of cheaters would probably quickly increase to equilibrium, with the level of opportunity provided by careless brooders.

This reasoning suggests that the first brooder in a population of predominantly nonbrooders must have been adapted for a high level of paternity assurance. Members of the subfamily Lethocerinae (*17*) (nonbrooding giant water bugs) are believed to have a common ancestry with the Belostomatinae. Modern *Lethocerus* males repeatedly mate an individual female and may guard her between bouts of copulation (*18*). Multiple mating and guarding of an appropriated female by male insects apparently is an adaptation to prevent sperm displacement from subsequent matings by another male (*8*, *19*). It is not difficult to envision a pattern of alternating bouts of copulation and oviposition evolving from the lethocerine system, and under suitable ecological conditions (such as absence of, or limited, emergent vegetation, intense predation, and egg parasitism), it might have been advantageous for females to begin depositing their eggs on their mates' backs, and for males to begin brooding them.

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13. In replicate five, the second male (No. 17) died before the eggs on its back hatched. Twenty-one of 99 eggs hatched, and only six nymphs were of the wild-type phenotype. This anomaly suggests that the wild type (++) male (No. 17) experienced sexual dysfunction (perhaps related to senescence) at the time of the male's pairing with the previously mated ++ female. This event was significant in that it further dramatized male risk, and demonstrated the utility of previously stored sperm as a female backup system.
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Dr. Guthrie and *Felis domesticus* Or: Tripping over the Cat

Abstract. *The principal reactions described in Guthrie and Horton's classic learning monograph appear to have been caused by the mere presence of the experimenters. Neither escape nor food reinforcement is necessary for the establishment of such responses. They are species-typical "greeting" reactions, readily elicited by the sight of human observers.*

For almost half a century, most research in instrumental (operant) conditioning has been conducted without regard for the natural behavior of the animals used as subjects (*1*, *2*). A recurrent consequence of this tradition has been the failure to recognize species-typical reactions that are critical in certain learning situations. The pigeon's "operant" key-pecking response, for example, was not identified as a simple grain-pecking reaction, and a generation of investigators therefore believed that they had taught tens of thousands of pigeons, individually, how to peck. The implications of this error have been only partially explored (*3*).

Failure to recognize the common avian peck was not an isolated aberration (*4*). We report here the discovery of another such oversight, this time from one of the two classic studies of learning in domestic cats (*5*, *6*).

In the puzzle-box experiments of Guthrie and Horton (*5*), cats were photographed and observed as they learned to escape from a small, glass-fronted chamber. A conspicuous escape door in the front of the chamber could be opened by jostling a slender, vertical rod about 1 foot away. The ability of cats to master so simple a task was not in question, but great importance was attached to the manner in which their "learning" was expressed. The animals' responses were described as highly stereotyped, with long series of movements repeated "in remarkable detail" from trial to trial. Several different reactions were recorded, but the commonest was that shown in Fig. 1. The stereotypy of this behavior was interpreted as evidence for a process of learning by stimulus-response contiguity (*5*, *7*), and the tracings shown in Fig. 1 were widely reprinted in support of that conclusion. Elsewhere, the same stereotyped reactions were cited as evidence of "superstitious" operant conditioning (*8*).

Neither interpretation is warranted.

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