

Learning in intimate connections: Conditioned fertility and its role in sexual competition

Michael Domjan, PhD*, Michael J. Mahometa, PhD and
R. Nicolle Matthews, PhD

Department of Psychology, The University of Texas at Austin, USA

Background: Studies of sexual conditioning typically focus on the development of conditioned responses to a stimulus that precedes and has become associated with a sexual unconditioned stimulus (US). Such a sexually conditioned stimulus (CS) provides the opportunity for feed-forward regulation of sexual behavior, which improves the efficiency and effectiveness of the sexual activity.

Objective and Design: The present experiments were conducted to provide evidence of such feed-forward regulation of sexual behavior in laboratory studies with domesticated quail by measuring how many fertilized eggs were produced by the female after the sexual encounter. During the conditioning phase, male and female quail received a conditioned stimulus paired with the opportunity to copulate with each other.

Results: Sexual conditioning increased the number of eggs that were fertilized as a consequence of copulation, especially if both the male and the female were exposed to the sexual CS. This conditioned fertility effect occurred with a range of CS durations and CS types. The conditioned fertility effect also occurred in situations involving sexual competition. When two males copulated with the same female, DNA fingerprinting showed that the male whose sexual encounter was signaled by a sexual CS was responsible for most of the resulting offspring. Sexual conditioning also reduced the first-male disadvantage in fertilization that occurs when two males copulate with the same female separated by several hours. Another significant finding was that sexual conditioning attenuated the usual drop in fertilization rate that occurs when the same male copulates with two females in succession.

Conclusion: These results show that sexual conditioning increases the number of offspring that are produced in both isolated male-female encounters and in situations that involve two males copulating with the same female or one male copulating with more than one female. By increasing fertilization rates, sexual conditioning can alter genetic transmission across generations and shape evolutionary change.

Keywords: *sexual conditioning; sexual learning; conditioned fertility; sexual competition; quail; pavlovian conditioning*

Wouldn't it be nice to be able to predict the outcome of a lottery drawing or when and where your old car is likely to break down? The ability to predict what will happen next enables organisms to mount coping strategies that can significantly improve their interactions with their physical and social environment (Domjan, 2005). The classic work of Pavlov (1927) uncovered a simple learning mechanism that serves such predictive functions. The reliable pairing of a new stimulus (the conditioned stimulus or CS) with a

biologically potent event (the unconditioned stimulus or US) allows the organism to anticipate and interact more effectively with the US. Because the CS signals the impending presentation of the US, Pavlovian conditioning is fundamentally a form of feed-forward regulation (Domjan, Cusato, & Villarreal, 2000). This feed-forward regulation can involve both behavioral and physiological adjustments.

Pavlovian conditioning has been studied for more than 100 years. However, during much of that time, the emphasis was on using Pavlovian conditioning as a paradigm to investigate how conditioned responses are learned and how a CS comes to be associated with a US. Although Pavlov and others were mindful of the

Current addresses: Michael J. Mahometa, Division of Statistics and Scientific Computation at the University of Texas at Austin, Austin, TX, USA. R. Nicolle Matthews, Clinical Department at the Judge Rotenberg Center in Canton, Canton, MA, USA.

biological advantages that accrue from learning conditioned responses (Culler, 1938; Pavlov, 1927), the functional significance of Pavlovian conditioning did not command much attention until the latter part of the twentieth century (Hollis, 1997).

In examining the functional significance of Pavlovian conditioning, one can focus on proximate mechanisms that operate on a relatively short time scale and involve only the immediate environment of the organism. Another approach is to focus on ultimate mechanisms that involve long-range, transgenerational, or evolutionary consequences. Although Pavlov was interested in both types of mechanisms, his work focused on proximate causes. In many of his experiments, dogs received a visual cue or CS paired with the presentation of food. With repetitions of such training trials, the dogs came to salivate when the CS was presented. Such conditioned salivation no doubt facilitated the digestion of the food, affording a proximate advantage. One may suggest that dogs with the ability to learn conditioned salivary responses were healthier and, therefore, had more offspring. But, such an inference would be entirely speculative. In most Pavlovian conditioning situations, evidence of functional significance is limited to proximate mechanisms. With few exceptions, the ultimate advantage of Pavlovian conditioning in increasing evolutionary fitness has remained out of the reach of empirical data.

A major advantage of studying sexual conditioning is that it allows examining not only the proximate functions of such learning but also its ultimate functions. Short-term improvements in sexual behavior resulting from Pavlovian conditioning may be seen in preparatory sexual responses and improvements in how efficiently the male and female interact during courtship and copulation. The ultimate biological function of sexual behavior is the production of offspring and, through those offspring, the transmission of genetic information to future generations. If short-term and long-term functions are related, one might expect that sexual Pavlovian conditioning will also result in the production of more offspring and, hence, increase reproductive fitness. The experiments reported in this article were conducted to test this hypothesis.

The experiments were conducted with domesticated quail (*Coturnix japonica*) because that species has been extensively used in studies of sexual conditioning (see Domjan & Akins, 2011, for a recent review). Copulation in quail involves the female squatting and remaining relatively still while the male grabs the back of the female's head, mounts on her back, and then makes cloacal thrusts to bring his cloaca in contact with that of the female for sperm transfer. When a male and female are first placed in a test arena, the male appears to take the initiative in chasing and grabbing the female. The male's approach response can be conditioned to a light or other localized stimulus that is paired with copulatory opportunity

(Burns & Domjan, 2000). Unlike the male, the female does not approach a sexually conditioned stimulus but she is more likely to squat if she encounters a male following a sexual CS, and females prefer locations where a male was previously housed (Gutiérrez & Domjan, 1997, 2011).

Previous studies have demonstrated that sexual conditioning significantly increases the proximal functionality of reproduction in quail. A frequently observed effect, initially reported by Domjan, Lyons, North, and Bruell (1986), is that sexually conditioned males initiate copulation with a female sooner than males that encounter a female without a sexual CS. This decreased latency to copulate allows signaled males to copulate first if a female is released in the company of two males (Gutiérrez & Domjan, 1996). Sexual conditioning also results in the release of greater quantities of sperm (Domjan, Blesbois, & Williams, 1998), probably because the presentation of a sexual CS to males causes anticipatory cloacal gland contractions (Holloway, Balthazart, & Cornil, 2005).

Another major aspect of increased proximal functionality is an increase in the efficiency of copulatory behavior. Copulatory efficiency may be measured by calculating how often a male's grab and mount responses end in cloacal contact. If the male is not effective when he first grabs and tries to mount the female, the female is apt to throw him off and run away. Ordinarily, many of the copulatory attempts of the male end without cloacal contact. However, the efficiency of the male's behavior can be substantially improved by presenting a sexually conditioned stimulus (Mahometa & Domjan, 2005). Interestingly, the increase in copulatory efficiency is not just a function of the male's behavior but also the willingness of the female to squat when approached by a male. Measures of copulatory efficiency are highly correlated with the duration of the female's squatting response (Domjan, Mahometa, & Mills, 2003).

If Pavlovian conditioning also improves the ultimate functions of sexual behavior, then male–female pairs that copulate following exposure to a sexually conditioned stimulus should produce more offspring than male–female pairs that copulate in the absence of such Pavlovian signaling. This prediction was first tested and confirmed in a study of sexual conditioning with gourami fish by Hollis, Pharr, Dumas, Britton, and Field (1997). The conditioned fertility effect was subsequently confirmed in coturnix quail by Adkins-Regan and MacKillop (2003) and Mahometa and Domjan (2005).

The present experiments were conducted to explore the range of circumstances in which the conditioned fertility effect may be observed in quail. In particular, we were interested in testing procedures that captured some of the complexity of the natural ecology of quail. The first set of experiments involved evaluating the effect of various aspects of the CS in tests of an individual male copulating

with an individual female. The second set of experiments explored the role of the conditioned fertility effect in more complex social situations involving some form of sexual competition (two males copulating with one female or one male copulating with two females). Since the emphasis in the present series of experiments is on the fertility consequences of sexual conditioning, we will rarely report behavioral data such as the latency to initiate copulation or copulatory efficiency. Details about these measures (which showed results in line with our previously published work) and other aspects of the experiments are available in Mahometa (2006) and Matthews (2005).

CS variables in the conditioned fertility effect

Conditioned fertility with a range of CS durations

A major variable in studies of Pavlovian conditioning is the duration of the CS. In general, conditioned responding is less likely with procedures that employ longer CS durations. This function was first examined in detail in eyeblink conditioning (e.g. Schneiderman & Gormezano, 1964). Studies of the effects of the CS duration in sexual conditioning have yielded a more complex pattern of results. Sexually conditioned approach responding declines with longer CS durations but measures of conditioned locomotor behavior show increases (Akins, Domjan, & Gutiérrez, 1994; see also Burns & Domjan, 2001). However, in these experiments, only male quail received sexual conditioning. Since female quail appear to be slower than males in becoming sexually aroused, sexual conditioning may be more successful in females with longer CS durations. The present experiment was conducted to test this hypothesis.

Thirty-six pairs of male and female quail served in the experiment. The birds were housed on either side of a large arena (71 cm high \times 122 cm wide \times 122 cm deep) that was divided in half by a plywood wall. A door in the middle of the wall could be raised giving the birds an access to each other. A small green light was positioned in each compartment above the door and was used as the conditioned stimulus. Fifteen conditioning trials were conducted, each on a separate day. For male quail, the CS light was always presented for 30 sec before the door was opened giving the male access to the female. For female quail, the duration of the CS light depended on group assignment. Independent groups received CS durations of 30 sec, 10 min, or 20 min before each copulatory opportunity. Each copulatory opportunity lasted 5 min, after which the male and female were returned to their respective compartments.

At the completion of the 15 days of training, all subjects were individually housed for 15 days (to void the females of sperm). The birds were then placed back in the testing chambers for a 5-min copulatory test. For testing,

a set of male–female pairs were randomly selected to serve in an un signaled control group, which did not receive any CS prior to the 5-min copulatory test (and, therefore, could thus not predict the upcoming copulatory opportunity). All other male–female pairs received the CS duration they were trained with. After the CS test trial, the birds were again individually housed. Eggs were collected from the females for 10 days and then examined for evidence of fertilization.

The results of the fertility assessments are summarized in Fig. 1 as a function of the CS duration that was used during the conditioning and test trials for the females. As we have observed previously (e.g. Mahometa & Domjan, 2005), the control group that did not receive a Pavlovian signal on the test day showed a very low rate of fertilization (less than 5%). In contrast, each of the other groups showed significantly higher fertilization rates. However, there was no evidence of higher rates of fertilization in females that were conditioned with longer CS durations. These results confirm that Pavlovian signaling increases the rate of fertilization and extent this finding in two important ways. First, the effect was observed whether or not the duration of the CS presented to the male was the same as the CS duration used with the female. Second, the salutary effects of sexual conditioning on rate of fertilization of the eggs were observed over a wide range of CS durations used with female quail (30 sec to 20 min).

Conditioned fertility with a naturalistic CS

In the preceding study, the CS was a small green light presented before the male and female quail received access to each other for copulation. The fact that such a sexual CS can increase fertilization success clearly shows that fertilization is subject to modification by learning

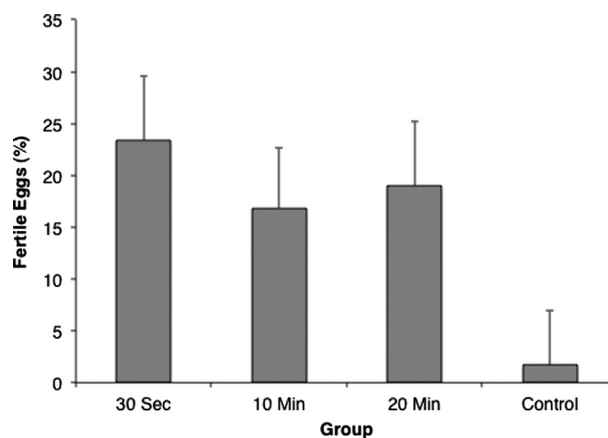


Fig. 1. Rate of fertilization of eggs in quail (mean + S.E.) as a function of the CS duration used during conditioning and testing for female quail. Males received a uniform CS duration of 30 sec. The control group received sexual conditioning but was not exposed to the CS before the copulatory test trial.

procedures. However, the significance of this finding for naturally occurring sexual behavior may be questioned since it is unlikely that quail would encounter a small light just before they had a chance to copulate in their natural habitat. In the natural environment, the pairings of a CS and an US are not arranged by an investigator in a laboratory coat. Rather, pairings occur because the CS is a small component of the US or occurs early in a causal chain of events that reliably leads to the US (Domjan, 2005).

Coturnix quail are ground birds and live in grassy areas. Therefore, before a male is close enough to a female to copulate with her, he is apt to see just part of her body through the grass. The most likely visible body part is the head, and perhaps some portion of the neck. In fact, studies with taxidermic models that included different parts of a female's body indicated that the head and neck feathers of a female are particularly salient for males (Domjan & Nash, 1988). Therefore, sexual conditioning in the natural habitat of quail may involve cues of the female's head as the conditioned stimulus, followed by access to the entire female as the male approaches the head cues. Consistent with this suggestion, cues of a female's head have been found to be especially effective in studies of sexual conditioning in quail (Domjan, Cusato, & Krause, 2004).

The present experiment was conducted to measure the fertility effects of sexual conditioning in quail when a naturalistic CS was used rather than an arbitrary light. The naturalistic CS consisted of the head and limited neck feathers of a female quail positioned on a 10-cm vertical dowel. For a different group of birds, the CS was an object of similar size and shape but made of terry-cloth. When not in use, the CS objects were covered with an opaque cover that could be raised by the experimenter. To prevent the birds from pecking or attempting to mount the CS objects, the objects were covered with a clear plastic cup when visible. Ten conditioning trials were conducted for each group of birds. For each trial, the CS was presented for 30 sec to both the male and female, followed by 5 min of copulatory access. After the conditioning trials, the birds were moved to their home cages for 10 days to void the females of sperm. A single copulatory test was then conducted to assess fertilization success.

For testing, an equal number of male–female pairs from each CS training condition were placed in an unsignaled control group. These birds did not receive a CS prior to copulatory opportunity on the test day. The remaining male–female pairs were presented with the CS that they had been conditioned with prior to a single 5-min copulatory opportunity. The birds were then moved to the colony room where they were individually

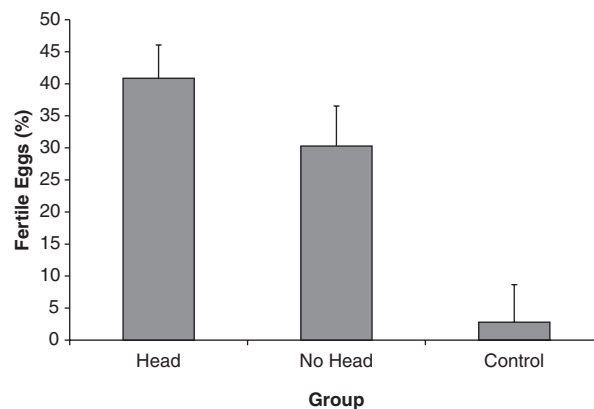


Fig. 2. Rate of fertilization of eggs in quail (mean + S.E.) for male–female pairs that were conditioned and tested with a CS that consisted of the taxidermic head of a female or a comparable terrycloth object. Birds in the control group also received sexual conditioning but were tested without exposure to the CS.

housed and where eggs were collected from the females to assess rates of fertilization following the test copulations.

Fertilization rates for the different test groups of birds are presented in Fig. 2. As seen previously, very low rates of fertilization occurred in the unsignaled control group. The highest rates of fertilization occurred for birds that were conditioned with the head CS. However, the difference in fertilization rate between the head CS and terrycloth CS groups was not statistically significant. These results extend the conditioned fertility effect to Pavlovian procedures that involve a more naturalistic CS. The present results show that a naturalistic CS is not necessary to obtain the conditioned fertility effect and may not even increase the size of the effect.

Conditioned fertility with contextual cues

In each of the preceding experiments, the CS was a discrete, spatially localized visual cue. Although not much is known about the natural ecology of coturnix quail, evidence suggests that females maintain territories where they eventually establish a nest and incubate their eggs. In contrast, males are more transitory, moving across female territories. Under these conditions the contextual cues of a female's territory may become associated with copulatory opportunity. Thus, sexual conditioning in the wild may involve contextual cues.

The next question we asked is whether a more complex and diffuse contextual cue could produce the conditioned fertility effect. As a part of this experiment, we also evaluated which partner (the male or the female) has to be signaled for the conditioned fertility effect to occur. Using a green light CS, we previously found that the conditioned fertility effect only occurs if both the male and the female are exposed to the Pavlovian CS prior to a test copulation (Mahometa & Domjan, 2005). In contrast to this outcome, Adkins-Regan and MacKillop (2003)

reported significantly increased fertility rates in a study that involved only signaling the male or the female.

Contextual cues in the present experiment were created by placing the birds in one of two distinctive experimental chambers (71 cm high \times 122 cm wide \times 122 cm deep). One of these chambers was painted entirely white, and the other was painted entirely brown. To further help distinguish the two contexts, one chamber was housed in the colony room with relatively high levels of ambient noise, whereas the other was housed in a quiet test room. Both chambers were divided down the middle by a sheet of plywood – creating separate male and female compartments. The divider had a door in the middle that the birds could pass through when raised.

Training for all male–female pairs lasted 10 days. Each day, all pairs of birds received one CS+ trial and one CS– trial. On CS+ trials, all the birds were placed in one of the contexts for 5 min (the CS duration) and then received 5 min of copulatory opportunity. On CS– trials, the birds were placed in the alternate context for 5 min after which the center door was raised but there was no sexual partner on the other side. This design effectively trained a specific context as the CS that signaled copulation. The alternate context was equally familiar but was not associated with sexual reinforcement. Which context (white or brown) served as CS+ and CS– was counterbalanced across participants.

Unlike the prior experiments, this time the birds did not remain in distinct copulatory pairs for training and testing. Rather, copulatory partners were rotated across trials. This was done so that encounter with a novel partner on the test trial would not be disruptive. After 10 days of training, the birds were housed individually for 15 days to void the females of sperm. On the day of testing, the birds were sorted into one of four test groups. The groups were differentiated in terms of which sexual partner (male or female, both, or neither) was exposed to the CS+ context prior to the test copulation, which lasted 5 min. After the test copulation, the birds were housed individually in the main colony room, and females had their eggs collected to determine rates of fertilization.

On the last day of training, we measured how long males took to cross over to the other side of the experimental chamber. Not surprisingly, males had very low latencies to pass through the raised door at the time of the US presentation in the CS+ context. They were significantly slower to pass through the partition door in the CS– context. These results indicate that the males learned to distinguish the two contexts and that one had become a signal for sexual reinforcement whereas the other had not.

The results of the fertility measurements are displayed in Fig. 3. The highest rates of fertilization occurred when copulation was signaled by the CS+ context for both the

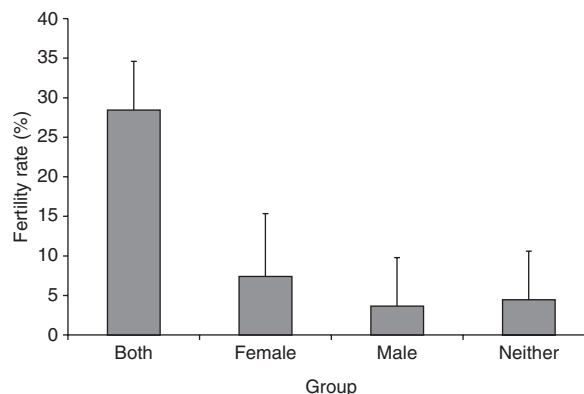


Fig. 3. Rate of fertilization of eggs in quail (mean + S.E.) for male–female pairs in which copulation was signaled by a contextual cue for both sexual partners, only the female, only the male, or neither.

male and the female. In contrast, the lowest rates of fertilization were observed when copulation was not signaled for either sexual partner. Interestingly, signaling copulation only for the female or only for the male did not produce more fertilization than what was observed in the control condition.

The present findings extend the conditioned fertility effect to situations in which contextual cues are used to signal copulatory opportunity. Since contextual cues are likely to be involved in sexual conditioning in the natural ecology of quail, these results suggest that conditioned fertility effects probably also occur under natural conditions.

The present results indicate that for the conditioned fertility effect to occur, both sexual partners have to receive the Pavlovian signal prior to the copulation. We previously obtained similar results in a study that used a green light as the CS in sexual conditioning (Mahometa & Domjan, 2005). This outcome contrasted with the findings of Adkins-Regan and MacKillop (2003), who reported conditioned fertility effects when copulation was signaled for only the male or only the female. One possible reason for this discrepancy was that Mahometa and Domjan (2005) used a localized light as the CS whereas Adkins-Regan and MacKillop (2003) used contextual cues in their experiments. However, the present findings suggest that this was not the critical factor. Rather, the current findings encourage exploring other procedural variables that differentiated the Adkins-Regan and MacKillop (2003) experiments from those of Mahometa and Domjan (2005).

Conditioned fertility and sexual competition

The preceding experiments and related previously published research amply demonstrate that sexual conditioning can increase fertilization success and the numbers of offspring that result from a sexual encounter. In all of

these experiments, one male interacted with one female after exposure to a Pavlovian signal as compared to the absence of such signaling. Given these results, what would happen in more complex social situations that involve sexual competition? How might Pavlovian signaling influence reproductive outcomes in situations where more than one male attempted copulations with the same female or the same male attempted to inseminate more than one female? The experiments reported in this section were conducted to explore these scenarios.

Sperm competition with a 15-min temporal delay

The standard definition of sperm competition suggests that sperm from more than one male compete to fertilize the egg of a single female during a single reproductive cycle (Birkhead & Møller, 1992; Birkhead & Pizzari, 2002). Sperm with advantageous morphological traits such as large heads (LaMunyon & Ward, 1999) and long tails (Simmons & Kotiaho, 2002) will traverse the oviduct more quickly and arrive first at the ova. In most avian species, including the coturnix quail, sperm require 15 min to travel from cloaca to infundibulum. An egg is available for fertilization for less than 20 min before the first layer of calcium surrounds the follicle making it impenetrable to sperm (Birkhead & Møller, 1992). This leaves little time for pure sperm competition in the form of a direct race between two sperm from entry at the cloaca to fertilization at the infundibulum. For a male to fertilize a greater proportion of the eggs than his competitors, he must either copulate closer to the fertilization window (Lewis, 2004; Schwagmeyer & Parker, 1990) or provide greater quantities of sperm (Birkhead, Hunter, & Pellatt, 1990).

Typically, when two males mate in quick succession with the same female, they transfer similar quantities of sperm and share equally in paternity of the offspring (Birkhead & Møller, 1992). Potentially, a Pavlovian CS can bias the outcome of sperm competition if copulation is signaled for one of two males mating with the same female. If Pavlovian conditioning provides an advantage by increasing the likelihood of fertilization, males that are able to anticipate copulatory opportunity should sire more offspring than control males. We tested this prediction in a study that was originally reported in Matthews, Domjan, Ramsey and Crews (2007). Paternity was determined using microsatellite-based DNA analysis, which was selected to decrease type II identification errors (Hanotte, Bruford, & Burke, 1992).

The sexual conditioning procedure was similar to that of the preceding experiment and employed contextual cues as conditioned stimuli. As in that study, two distinctive contexts were used. One of the contexts had a flat floor and green walls and was 2 in. off the floor of a noisy room that housed other quail; the other chamber was painted white, had a tilted floor, and was located in

an quiet test room 3 ft off the floor. An opaque barrier divided each chamber in half to create separate compartments for the male and the female. The barrier contained a small door, which could be raised to allow contact between the birds, as needed.

The male and female quail were first habituated to both contexts and then received conditioning trials that involved exposure to the context CS for 1 min followed by raising the center door to provide access to the other side of the chamber for 5 min. For males, one of the contexts (counterbalanced across birds) was designated as the CS+ and exposure to this context was followed by copulatory opportunity on each of five conditioning trials. Equal exposure to the alternate context (the CS−) was provided without sexual reinforcement to establish a control context. For female participants, both contexts were paired with access to a male so that the females would receive signaled copulations during the test trial regardless of the context in which the test copulations occurred. The intent of these procedures was to establish Pavlovian signaling as an experimental variable only for the male participants.

Following sexual conditioning, females were housed individually for 14 days to void them of sperm before the sperm competition test. For the competition test, each female was mated with two males in succession, separated by a 15-min delay between the males. For one of the males, the test copulation was preceded by placement in its CS+ context. For the other male, the test copulation was preceded by placement in its control context. (Because of their conditioning history, both contexts predicted copulatory opportunity for the females.) As during the conditioning trials, CS exposures during the competition test were 1 min, and copulation periods were 5 min. Whether the signaled or control male copulated with a given female first was counterbalanced across female participants.

Males that were placed in the context that was predictive of access to a female were quicker to initiate cloacal contact ($M = 3.7$ sec, $SE = 7.0$ sec) than males that were placed in the control context ($M = 19.1$ sec, $SE = 3.6$ sec) prior to the test copulation. However, no differences in the total frequency of cloacal contacts were found between males copulating in the signaled and control contexts. This suggested that the difference in rates of fertilization that we obtained (see below) were not due to a difference in the number of copulations in one context versus the other.

Following 10 days of egg collection and 5 days of incubation, all eggs were opened for examination of embryonic development. Of the 78 eggs laid by the females, 39 eggs contained embryonic tissue. Genetic analysis indicated that 28 of these (72%) were sired by the signaled males and 11 were sired by the control males. Evaluated in another manner, 10 of the 14 females

produced more eggs fertilized by the signaled male than the control male.

These findings demonstrated for the first time that prior individual experience in the form of Pavlovian conditioning can have a significant role in sperm competition. When two males mated with the same female, the one able to predict (and presumably prepare for) copulatory opportunity was able to fertilize more of the eggs and sire more of the offspring. These finding shows that learning and individual past experience can bias genetic transmission and the evolutionary changes that result from sexual competition.

Sperm competition with a 5-h temporal delay

In the preceding study, two males copulated with the same female within a relatively short period. Under these conditions and in the absence of Pavlovian signaling for either male, one would expect that approximately half the offspring would be sired by each male. Indeed, in a control experiment conducted in a manner very similar to the preceding one, we found that the first and second male were responsible for 55 and 45% of the resulting fertilized eggs, respectively, if neither of them received a Pavlovian signal. This statistically equal split is dramatically altered if a substantial delay (e.g. 5 h) is introduced between the two males. Such a long delay produces a significant change in paternity due to the exponential death rates of sperm in the sperm storage tubules (Colegrave, Birkhead, & Lessells, 1995), which provide a paternity advantage to the second male.

The next experiment was designed to evaluate sexual competition in coturnix quail when two males copulated with the same female with a 5-h separation between the two copulations. In addition, we evaluated whether the disadvantage suffered by the first male under these conditions could be overcome by signaling his copulatory opportunity.

As in the preceding experiment, a single female was permitted to copulate with two males. However, this time, the two copulatory periods were separated by a 5-h delay. Two groups were tested. For the delay control group, the test copulations for both the first and the second male occurred in a context that had not been previously associated with sexual reinforcement. In contrast, for the delay signal group, the first male received access to the female in a context that was previously associated with sexual reinforcement whereas the second male did not receive such Pavlovian signaling. Details of the conditioning and test procedures were as in the preceding experiment, with all test copulations signaled for the females.

As in the preceding experiment, males that were placed in the context predictive of access to a female were quicker to initiate cloacal contact responses ($M = 5$ sec, $SE = 0.3$) than males that were placed in the control context prior to the test copulation ($M = 19$ sec, $SE = 4.8$). However, there

were no significant differences in copulatory efficiency or the number of grabs, mounts, and cloacal contacts that were made by males in the signal versus control contexts. Therefore, differences in paternity could not be attributed to differences in copulation frequency.

Females in the delay control group produced 35 fertilized eggs whereas females in the delay signal group produced 28 fertilized eggs. More importantly, the proportion of eggs fertilized by the first and second male was drastically different for the two groups. The delay control group showed the usual first-male disadvantage, with the first male being responsible for only 26% of the fertilized eggs and the second male being responsible for 74% of the fertilizations. This first-male disadvantage was significantly ameliorated in the delay signal group, where the first male fertilized 43% of the eggs and the second male fertilized 57%.

These findings confirm the assumption that Japanese quail are similar to other avian species in showing a first-male disadvantage when two males copulate with the same female with a considerable delay between the copulations. However, the disadvantage that comes with being the first male can be attenuated if the first male copulates in a sexually conditioned context. Together with the previous study, these results support the conclusion that Pavlovian conditioning can increase the success of males in sexual competition whether or not that competition involves a substantial interval between access to the female for the first and second male.

Sperm allocation in copulations with two females

As we saw in the preceding experiments, a signaled male can gain an advantage in sexual competition by being more successful in fertilizing a female's eggs when more than one male copulates with the same female. Males can also gain an advantage by inseminating more than one female. The next experiment was conducted to determine if sexual conditioning changes the outcome in such a sperm allocation situation.

Repeated matings occur in most species and provide a mechanism for increasing insemination, which can then be used to increase the amount of sperm provided to a single female or to multiple females (Lewis, 2004). Consecutive matings with the same female provide a slight increase in fertilization success, but the amount of sperm that is transferred declines exponentially with repeated copulations. Thus, males who mate several times before their sperm stores are replenished compromise their chance of reproductive success (Jones & Elgar, 2004). The faster a male can replenish his sperm stores, the faster copulations can resume at optimal levels. However, sperm replenishment requires time and metabolic resources. To preserve sperm for possible future copulations, males may not transfer all of their sperm in an initial copulation, leaving open the possibility that the

effectiveness of subsequent copulations may be altered by Pavlovian signaling.

In the present experiment, the same male birds copulated with two females presented in succession. In one test condition, access to the two females was provided 5 min apart, not allowing time for the replenishment of sperm. In the second test condition, access to the two females was provided with a delay of 5 h between them. Each male first participated in control tests, which were conducted prior to sexual conditioning so that the experimental chambers in which the copulations occurred were not yet associated with sexual reinforcement. Two weeks later, sexual conditioning was conducted as in the preceding experiments to establish one experimental chamber as a signal for sexual reinforcement while another chamber served as a familiar but not sexual context. Copulation tests were then conducted again with 5 min or 5 h separating the copulations with successive females. In these tests, either the first or the second female was presented in a context that had been sexually conditioned for the target male. (As in the preceding experiments, all of these test copulations were signaled for the female participant.) During all test sessions, each copulation period was terminated after the male achieved one cloacal contact with the female rather than after a fixed time period. This was done to make sure that there would be no differences in the frequency of cloacal contacts with the first and second female.

The results of the test sessions are presented in Table 1. When the males copulated with two females presented 15 min apart in the absence of Pavlovian signaling, 24% of the eggs laid by the first female were fertilized but only 6% of the second female's eggs were fertilized. This reflects the common sperm-depletion effect or some kind of a behavioral satiation effect. Having just copulated with the first female, the males may not have been as efficient in making cloacal contact and transferring sperm to the second female. A sexually CS that signaled

Table 1. Percentage of fertilized eggs laid by two females inseminated by the same male quail at two different delays between the copulations. Tests were conducted in which the male was not signaled or was signaled when copulating with the first or second female

	1st Female (%)	2nd Female (%)
15-min delay		
No signal	24	6
Signal for 1st female	41	5
Signal for 2nd female	23	27
5-h delay		
No signal	27	24
Signal for 1st female	40	20
Signal for 2nd female	21	37

either the first or the second female served to increase fertilization rates. In the case of the first female, introducing a Pavlovian CS increased fertilization rates from 24 to 41%. In the case of the second female, the increase due to Pavlovian signaling was from 6 to 27%. Thus, the normally low rate of fertilization of the second female could be substantially increased by presenting a sexually CS when the second female was introduced. Given the brevity of the Pavlovian signal (1 min), it is unlikely that the increase in fertilization of the second female reflected the induction of spermatogenesis. Rather, the Pavlovian signal may have improved the efficiency of cloacal contact, sperm release, or sperm transfer.

When a 5-h delay separated copulation with the first and second female, there was no evidence of sperm depletion or significantly lower fertilization rates for the first and second female. However, Pavlovian signaling still increased fertilization rates. When copulation was signaled for the first female, fertilization rates increased from 27 to 40%. When copulation was signaled for the second female, fertilization rates increased from 24 to 37%.

These results show that the conditioned fertility effect also occurs in sexual competition that involves sperm allocation between different females. The conditioned fertility effect increased fertilization success whether the male copulated with two females in relatively quick succession (15-min delay) or with greater temporal separation (5 h). In the present study, males selectively allocated sperm between two females, rather than using of all their sperm in the initial ejaculation. When a Pavlovian signal was provided, the male quail regulated sperm output so as to overcome the otherwise detrimental effects of repeat copulations. Increased fertility for the female copulating after a signal was evident whether she was the recipient of the first or second ejaculation.

Conclusion

Sexual selection is based on the evolution of traits that increase reproductive fitness. How learning or an individual's past experience may influence this process has not attracted much experimental attention. In particular, few have considered that Pavlovian conditioning may be a significant factor in sexual selection. Instead, physical and behavioral characteristics such as plumage (Calkins & Burley, 2003), tail length (Anderson, 1992), and parenting behavior (Putland, 2001) have been proposed as predictors of sexual selection. Each of these factors is likely to operate in unique ways in a particular species. In contrast to such idiosyncratic adaptations, the present experiments indicate that a common learning process, Pavlovian conditioning, can enhance reproductive success and provide a significant advantage in sexual selection.

The present experiments, together with prior studies with coturnix quail (Adkins-Regan & MacKillop, 2003; Mahometa & Domjan, 2005) and gourami fish (Hollis

et al., 1997), show that the presentation of a Pavlovian signal that was previously associated with access to a sexual partner significantly increases rates of fertilization and the numbers of offspring that result from a sexual encounter. The present experiments demonstrated that this conditioned fertility effect occurs in a wide range of circumstances. Of particular interest to us was whether the conditioned fertility effect can be observed under circumstances that are likely to occur in the natural habitat of the birds. Indeed, we found this to be the case. The conditioned fertility effect was no less robust when the stimulus used as the CS was a cue that is a natural precursor to copulation in the wild (the sight of a female's head). The conditioned fertility effect was also not attenuated by using contextual cues, which are more likely to accompany copulations in the natural environment than something like a small light.

Another feature of sexual behavior in nature is that females seek to increase their reproductive fitness by copulating with more than one male and males seek to increase their reproductive fitness by copulating with more than one female. Our experiments demonstrated that the conditioned fertility effect also operates in such multiple partner paradigms. If two males copulate with one female in close succession, ordinarily, they share equally in fertilizing the eggs subsequently laid by the female. However, if the copulation by one of the males is signaled for one of the males, that male ends up siring significantly more of the offspring. This improved success in sperm competition is particularly important if two males copulate with the same female separated by a 5-h interval. With such a long delay, most of the offspring are sired by the second male but presenting a Pavlovian CS to the first male eliminates this first-male disadvantage. Pavlovian signaling also prevents the typically decline in fertility that occurs when the same male copulates with two females in succession.

The robustness of the conditioned fertility effect in the present experiments is remarkable and demonstrates that the fecundity of organisms is not determined solely by their physiological and developmental readiness to reproduce. Rather, individual experience plays a major role. Furthermore, this individual experience does not involve non-specific socialization or extended practice with components of courtship and copulation. All of the birds in our experiments had the same laboratory rearing history prior to the critical conditioned fertility tests. In addition, in each experiment the different groups of birds had the same number of prior copulatory experiences and received the same type of sexual conditioning. What differentiated one group from another was the presence or absence of the sexually CS that all of the birds previously learned about. Thus, the conditioned fertility outcome in each experiment reflected the specific memories (and accompanying physiological and behavioral

changes) that were activated by the Pavlovian CS. These results indicate that reproductive fitness is determined by the individual learning history of each sexual partner and how that learning history is activated by the specific environment in which the copulations take place. By altering reproductive fitness, these mechanisms of learning and memory can influence genetic transmission and evolutionary change.

Acknowledgements

The research was supported by Grant 39940 from the National Institute of Mental Health.

Conflict of interest and funding

The authors have not received any funding or benefits from industry or elsewhere to conduct this study.

References

- Adkins-Regan, E., & MacKillop, E.A. (2003). Japanese quail (*Coturnix japonica*) inseminations are more likely to fertilize eggs in a context predicting mating opportunities. *Proceedings of the Royal Society of London, Series B*, 270, 1685–1689.
- Akins, C.K., Domjan, M., & Gutiérrez, G. (1994). Topography of sexually conditioned behavior in male Japanese quail (*Coturnix japonica*) depends on the CS-US interval. *Journal of Experimental Psychology: Animal Behavior Processes*, 20, 199–209.
- Anderson, M. (1992). Female preference for long tails in lekking Jackson's widowbirds: Experimental evidence. *Animal Behaviour*, 43(3), 379–388.
- Birkhead, T.R., Hunter, F.M., & Pellatt, J.E. (1990). Sperm competition in the zebra finch, *Taeniopygia guttata*. *Animal Behaviour*, 38(6), 935–950.
- Birkhead, T., & Møller, A.P. (1992). *Sperm competition in birds: Evolutionary causes and consequences*. London: Academic Press.
- Birkhead, T.R., & Pizzari, T. (2002). Post-copulatory sexual selection. *Nature Reviews Genetics*, 3, 262–273.
- Burns, M., & Domjan, M. (2000). Sign tracking in domesticated quail with one trial a day. Generality across CS and US parameters. *Animal Learning & Behavior*, 28, 109–119.
- Burns, M., & Domjan, M. (2001). Topography of spatially directed conditioned responding: Effects of context and trial duration. *Journal of Experimental Psychology: Animal Behavior Processes*, 27, 269–278.
- Calkins, J.D., & Burley, N.T. (2003). Mate choice for multiple ornaments in the California quail, *Callipepla californica*. *Animal Behaviour*, 65, 69–81.
- Colegrave, N., Birkhead, T.R., & Lessells, C.M. (1995). Sperm precedence in zebra finches does not require special mechanism of sperm competition. *Proceedings of the Royal Society of London B*, 259, 223–228.
- Culler, E.A. (1938). Recent advances in some concepts of conditioning. *Psychological Review*, 45, 134–153.
- Domjan, M. (2005). Pavlovian conditioning: A functional perspective. *Annual Review of Psychology*, 56, 179–206.
- Domjan, M., & Akins, C.K. (2011). Applications of Pavlovian conditioning to sexual behavior and reproduction. In T.R. Schachtman, & S. Reilly (Eds.), *Associative learning and*

- conditioning theory: Human and non-human applications* (pp. 507–531). New York: Oxford University Press.
- Domjan, M., & Nash, S. (1988). Stimulus control of social behavior in male Japanese quail (*Coturnix coturnix japonica*). *Animal Behaviour*, 36, 1006–1015.
- Domjan, M., Blesbois, E., & Williams, J. (1998). The adaptive significance of sexual conditioning: Pavlovian control of sperm release. *Psychological Science*, 9, 411–415.
- Domjan, M., Cusato, B., & Krause, M. (2004). Learning with arbitrary versus ecological conditioned stimuli: Evidence from sexual conditioning. *Psychonomic Bulletin & Review*, 11, 232–246.
- Domjan, M., Cusato, B., & Villarreal, R. (2000). Pavlovian feed-forward mechanisms in the control of social behavior. *Behavioral and Brain Sciences*, 23, 235–249.
- Domjan, M., Lyons, R., North, N.C., & Bruell, J. (1986). Sexual Pavlovian conditioned approach behavior in male Japanese quail (*Coturnix coturnix japonica*). *Journal of Comparative Psychology*, 100, 413–421.
- Domjan, M., Mahometa, M., & Mills, A.D. (2003). Relative contributions of the male and female to sexual behavior and reproductive success in the Japanese quail (*Coturnix japonica*). *Journal of Comparative Psychology*, 117, 391–399.
- Gutiérrez, G., & Domjan, M. (1996). Learning and male–male sexual competition in Japanese quail (*Coturnix japonica*). *Journal of Comparative Psychology*, 110, 170–175.
- Gutiérrez, G., & Domjan, M. (1997). Differences in the sexual conditioned behavior of male and female Japanese quail (*Coturnix japonica*). *Journal of Comparative Psychology*, 111, 135–142.
- Gutiérrez, G., & Domjan, M. (2011). Conditioning of sexual receptivity in female quail: Measures of conditioned place preference. *Behavioural Processes*, 87, 268–273.
- Hanotte, O., Bruford, M.W., & Burke, T. (1992). Multilocus DNA fingerprints in gallinaceous birds: General approach and problems. *Heredity*, 68, 481–494.
- Hollis, K.L. (1997). Contemporary research on Pavlovian conditioning: A “new” functional analysis. *American Psychologist*, 52, 956–965.
- Hollis, K.L., Pharr, V.L., Dumas, M.J., Britton, G.B., & Field, J. (1997). Pavlovian conditioning provides paternity advantage for territorial male blue gouramis (*Trichogaster trichopterus*). *Journal of Comparative Psychology*, 111, 219–225.
- Holloway, K.S., Balthazart, J., & Cornil, C.A. (2005). Androgen mediation of conditioned rhythmic cloacal sphincter movements in Japanese quail (*Coturnix japonica*). *Journal of Comparative Psychology*, 119(1), 49–57.
- Jones, T.M., & Elgar, M.A. (2004). Male age, sperm age and mating history on fecundity and fertilization success in the hide beetle. *Proceedings: Biological Sciences*, 271(1545), 1311–1318.
- LaMunyon, C.W., & Ward, S. (1999). Evolution of sperm size in nematodes: Sperm competition favours larger sperm. *Proceedings: Biological Sciences*, 266(1416), 263.
- Lewis, S. (2004). Multiple mating and repeated copulations: Effects on male reproductive success in red flour beetles. *Animal Behaviour*, 67(4), 799–804.
- Mahometa, M.J. (2006). *Adaptive significance of Pavlovian conditioning on reproductive behavior and fertility in Japanese quail (Coturnix japonica)*. Unpublished doctoral dissertation, University of Texas at Austin.
- Mahometa, M.J., & Domjan, M. (2005). Classical conditioning increases reproductive success in Japanese quail, *Coturnix japonica*. *Animal Behaviour*, 69, 983–989.
- Matthews, R.N. (2005). *Pavlovian conditioning alters reproductive fitness in sperm competition and sperm allocation paradigms*. Unpublished doctoral dissertation, University of Texas at Austin.
- Matthews, R.N., Domjan, M., Ramsey, M., & Crews, D. (2007). Learning effects on sperm competition and reproductive fitness. *Psychological Science*, 18, 758–762.
- Pavlov, I.P. (1927). *Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex*. Oxford, England: Oxford University Press.
- Putland, D. (2001). Has sexual selection been overlooked in the study of avian helping behavior? *Animal Behavior*, 62, 811–814.
- Schneiderman, N., & Gormezano, I. (1964). Conditioning of the nictitating membrane of the rabbit as a function of the CS-US interval. *Journal of Comparative and Physiological Psychology*, 57, 188–195.
- Schwagmeyer, P.L., & Parker, G.A. (1990). Male mate choice as predicted by sperm competition in thirteen-lined ground squirrels. *Nature*, 348, 62–64.
- Simmons, L.W., & Kotiaho, J.S. (2002). Evolution of ejaculates: patterns of phenotypic and genotypic variation and condition dependence in sperm competition traits. *Evolution*, 56(8), 1622–1631.

***Michael Domjan**

Department of Psychology
 1 University Station A8000
 The University of Texas at Austin
 TX 78712-0187, USA
 Email: domjan@psy.utexas.edu