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Egg investment is influenced by male attractiveness in the mallard

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Why females prefer to copulate with particular males is a contentious issue. Attention is currently focused on whether females choose males on the basis of their genetic quality, in order to produce more viable offspring¹. Support for this hypothesis in birds has come from studies showing that preferred males tend to

father offspring of better condition or with increased survivorship^{2–8}. Before attributing greater offspring viability to a male's heritable genetic quality, however, it is important to discount effects arising from confounding sources, including maternal effects. This has generally been addressed by comparing offspring viability from two different breeding attempts by the same female: one when offspring are sired by a preferred male, and one when offspring are sired by a less preferred male. However, here we show that individual female mallard (*Anas platyrhynchos*) lay larger eggs after copulating with preferred males and smaller eggs after copulating with less preferred males. As a result, females produced offspring of better body condition when paired with preferred males. After controlling for these differences in maternal investment, we found no effect of paternity on offspring condition. This shows that differences between half-sibs cannot always be attributed to paternal or maternal genetic effects.

Life-history theory predicts that females should alter their investment in a particular breeding attempt according to the likelihood of its success⁹. If preferred males provide any type of benefit to females, for example, better resources for breeding, individual females should alter their investment according to male attractiveness. This was first demonstrated by showing that females may increase their level of parental care when paired with preferred males^{10,11} (but see ref. 12). However, females may also alter their investment much earlier in reproduction; in some species, females lay more eggs when paired with preferred males than when paired with less preferred males^{13,14}. A problem arises if females instead alter their primary reproductive effort in ways that influence offspring quality rather than offspring quantity, for example, by laying larger eggs or increasing the nutritional content of eggs when paired with more attractive males. These effects would then have to be discounted before attributing all differences in offspring condition to viability genes inherited from the father. A recent study has shown that female zebra finches (*Taeniopygia guttata*), for example, alter their testosterone investment in eggs according to male attractiveness¹⁵. Testosterone is thought to influence early competitive ability in chicks¹⁶.

Female mallard show strong preferences for particular males, yet rear the precocial young on their own. Female preferences are clear from pairing displays performed throughout the autumn^{17–19}. Once pair bonds are formed in resident populations, they are maintained between years^{19,20}. Male mallard ($n = 20$) were ranked for attractiveness (see Methods). To ensure ranking was reliable, males were ranked twice with two separate groups of 20 female mallard and by a different observer on each occasion; male rank correlated significantly between the two groups of females (Spearman's rank correlation (r_s) = 0.61, $n = 20$, $P = 0.008$). Once male rank was established, we examined paternal and maternal effects on offspring traits by collecting three clutches of eggs from each of 16 females; an infertile control clutch, a clutch sired by a high-ranking male and a clutch sired by a low-ranking male. The two fertile clutches ($n = 32$

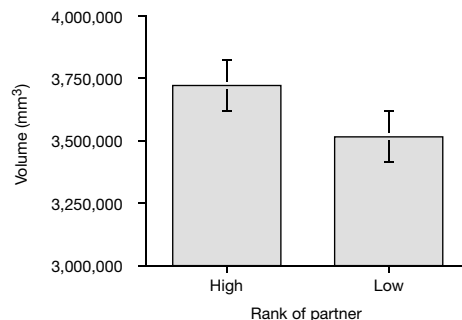


Figure 1 Mean egg volume (mm³) in high rank pairing and low rank pairing treatments (paired *t*-test, $t = 2.75$, $n = 16$ females, $P = 0.02$).

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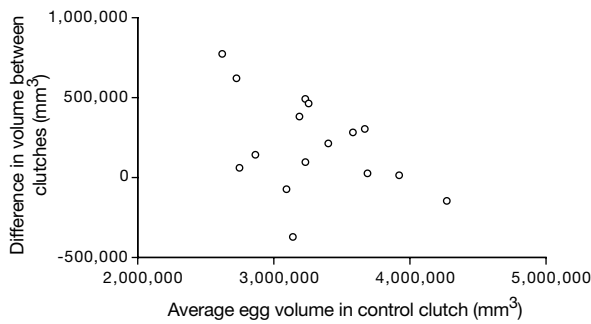


Figure 2 Females that laid smaller eggs in their initial control clutch were significantly more likely to lay eggs of different sizes when assigned to males of different attractiveness ($r_p = -0.435$, $n = 16$ females, $P < 0.05$).

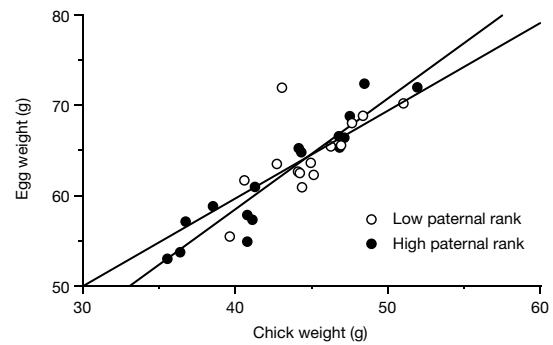


Figure 3 Relationship between mean egg weight and mean chick weight for each female ($F = 64.68$, d.f. = 14, $P < 0.001$) in high paternal rank and low paternal rank groups (analysis of covariance, $F_{\text{slopes}} = 2.62$, d.f. = 14, $P < 0.118$; $F_{\text{elevations}} = 0.16$, d.f. = 14, $P = 0.58$).

clutches, mean clutch size = 11.54, range 6–16) were incubated artificially under controlled conditions and chicks ($n = 200$) were monitored from hatching, through growth and development, until they themselves paired the following year.

We found that individual females laid significantly larger eggs when paired to more attractive males (Fig. 1). Egg size is a critical trait influencing fitness in birds^{21,22}; in the mallard, this is because young from larger eggs are better able to survive during the few days immediately after hatching²³. The differences in egg investment appeared to stem primarily from females that laid small eggs in their control clutch; females that initially laid larger eggs did not adjust egg size according to male rank (Fig. 2). However, the difference in average egg volume between clutches was unrelated to female condition (Pearson's correlation (r_p) = 0.021, $n = 16$, $P > 0.1$). There was no difference in the number of eggs laid by females for high- and low-ranking males (paired t -test, $t = 1.00$, degrees of freedom (d.f.) = 14, $P = 0.33$, mean number of eggs (\pm s.d.): high rank treatment, 12.6 ± 2.8 ; low rank treatment, 11.6 ± 2.7), suggesting that females were differing in their total investment rather than trading-off egg size and clutch size. This difference in egg investment resulted in females producing significantly heavier chicks when they were assigned to preferred males than when assigned to less preferred males (paired t -test, $t = 2.39$, d.f. = 14, $P = 0.03$; mean weight (\pm s.d.): high rank treatment, 45.0 ± 3.0 g; low rank treatment, 43.0 ± 4.8 g), though chicks did not differ in their skeletal size (paired t -test, $t = 0.28$, d.f. = 14, $P = 0.78$, mean tarsal length (\pm s.d.): high rank treatment, 49.6 ± 2.2 mm, low rank treatment, 49.5 ± 1.9 mm). This could not be explained by chicks that were fathered by preferred males being more likely to be male; there was no difference in the sex ratio of clutches fathered by high-

and low-ranking males (paired t -test, $t = 0.07$, $n = 16$ females, $P = 0.95$, mean proportion of males to females (\pm s.d.): high rank treatment = 0.47 ± 0.2 , low rank treatment = 0.47 ± 0.2). When we controlled for the difference in egg investment, there was no effect of male rank on chick weight (Fig. 3).

This is the first study to our knowledge to show that female investment, in terms of egg volume, may vary according to male attractiveness, even though males appear to provide no direct material benefits to females. It has been stated that differences in viability traits between half-sibs that share the same mother but that are sired by males of different attractiveness can be attributed to paternal genetic quality^{2,4,8}. The results of this study show that this may not be the case.

The question then is why do some females lay eggs of different sizes when assigned to preferred and less preferred males? Females may alter their egg investment in response to male attractiveness because attractive males are more likely to sire successful offspring. This could be adaptive whether the benefits of mating with preferred males are direct material benefits or indirect genetic benefits unrelated to viability, such as attractiveness genes. It is unclear why this should not be a beneficial strategy to all females, but it may be that females that initially laid smaller eggs were inherently of poorer quality and had the most to gain from increasing egg size with preferred males. Alternatively, females that initially laid larger eggs may not have been able to increase their egg size any further.

Therefore, we first examined the effect of paternal attractiveness on other measures of viability and male offspring attractiveness. We found no effect of egg size (all regressions, $P > 0.1$) or paternal rank on any other offspring traits that we recorded (Table 1). Instead, the

Table 1 Paired comparisons of traits between half-sibs sharing the same mother but fathered by preferred and less preferred males and hatching from first and second clutches.

Trait	Effect of male rank*				Effect of hatch date†			
	Mean (\pm s.d.)	t	P		Mean (\pm s.d.)	t	P	
Hatching success	49.5 ± 0.2 (high-rank male)	0.12	0.91		44 ± 25 (1st clutch)	1.34	0.20	
	48.5 ± 0.2 (low-rank male)				53 ± 22 (2nd clutch)			
Survival	82 ± 18 (high-rank male)	0.41	0.69		80 ± 22 (1st clutch)	0.39	0.71	
	86 ± 17 (low-rank male)				87 ± 12 (2nd Clutch)			
Attractiveness	3.8 ± 1.7 (high-rank male)	0.29	0.79		4.5 ± 1.2 (1st clutch)	3.21	0.03	
	4.0 ± 1.8 (low-rank male)				3.3 ± 1.8 (2nd clutch)			
	F_{slopes}	P	$F_{\text{elevations}}$	P	F_{slopes}	P	$F_{\text{elevations}}$	P
Growth rate	0.64	0.44	0.52	0.49	0.90	0.37	5.00	0.04
Moult development	0.09	0.76	0.29	0.59	0.28	0.61	8.76	0.01

* Hatch date balanced across treatments.

† Male rank balanced across treatments.

‡ Calculated from mean values for each clutch from each female.

single most important factor determining both viability traits and male attractiveness was hatch date (first versus second clutches) (Table 1). The onset of breeding is synchronous in mallard, but up to 96% of first clutches can be lost through depredation and females can lay up to four new clutches within a season¹⁹. Attractiveness in the mallard therefore appears to be an environmentally determined trait (see also ref. 24). Hatch date is primarily governed by nest depredation, which is mainly a chance event. Any genetic benefits to be gained from copulating with preferred males are therefore unlikely to become correlated with male attractiveness through future generations, including any ability by males to persuade females to lay larger eggs.

We then examined whether direct costs and benefits of pairing with preferred males may be important. Male mallard provide no paternal care and the benefits of pairing with particular males are not immediately apparent. However, there are at least two possibilities that could account for our findings. First, females prefer early hatched males. Dominance in wildfowl is often a consequence of pairing success²⁵. Early hatched chicks are the first males to pair and are therefore likely to be established as the most dominant males. Pair males defend transient feeding areas around females during the breeding season. Combined with their better body condition arising from earlier hatching, and the fact that early pairing increases their dominance status, such males are likely to be the most effective in defending a feeding area. This possibility is unlikely to explain our results directly as birds in this study were fed *ad libitum* and kept separately in pairs, but it may be one reason why females benefit from laying larger eggs for preferred males under natural conditions.

Second, the amount a female can invest in eggs may simply be a direct consequence of being paired to a particular male. There is a tendency for less preferred males to attempt more copulations with their partner than preferred males ($r_s = 0.68$, $n = 8$, $P = 0.07$), the majority of which are resisted by females. Unwanted copulation attempts in other species can influence female fecundity²⁶ and female longevity²⁷.

The results of this experimental study clearly show that females may invest differentially in eggs depending on the attractiveness of their partner. As a consequence, maternal investment can have an important influence on offspring quality. This study shows the fundamental importance of considering all maternal effects prior to attributing differences in offspring viability to paternal genetic effects in the future. □

Methods

Establishing experimental stock

Eggs were collected from wild-caught captive stock mallard that had been fed *ad libitum* from three months before egg laying to minimize any differences in female condition. Eggs were incubated artificially under standardized conditions. On hatching, chicks were reared in isolation from their parents and again fed *ad libitum* over the experimental period to minimize any differences in condition.

Ranking of males

The following spring, one experimental group of 20 males and two groups of 20 females were established. The first group of females was housed together with the males and allowed to pair freely. Over the pairing period, males were ranked for attractiveness to females by recording the number of females that directed pairing displays toward them. This is a reliable measure of female choice rather than the outcome of male–male competition^{17–19}. Once female preferences had been established, females were removed and housed individually until egg laying commenced. A second group of naive females were introduced into the experimental pen containing the previously ranked males. Female preferences were again recorded for the same males, by a second observer who had no previous knowledge of the males' initial ranking, to determine whether different females consistently prefer the same males.

Effects of male rank on offspring viability and female investment

Females were housed individually for about two weeks until they started laying. A control clutch of infertile eggs was collected to establish the average egg size for each female without the influence of male quality affecting female investment. Eggs were collected daily, measured and their volumes calculated using species-specific calculations²⁸.

The eight highest ranking males and the eight lowest ranking males were selected. Females were assigned one of these males for their next clutch; half of the females received a male of high rank and half a male of low rank. Pairs were housed together until females had finished laying an entire clutch. Eggs were collected each day and replaced with a chicken egg to induce females to lay a normal clutch. Once females had completed laying, males were removed. The chicken eggs were removed 7–10 days later to induce females to lay another clutch. A second male was then introduced to the female pen and the pair were left together throughout the female's second clutch. When re-laying occurred, females had been separated from their first male for at least 17 days, the longest period a female mallard has been recorded to store sperm^{19,29}, so that all offspring in the second clutch could be fathered only by the second male. For the second clutch, females previously paired with high-ranking males were paired to low-ranking males and *vice versa*, and the same procedure was followed for collection and incubation of the second clutch. Hence, two clutches were obtained from each female, one fertilized by a high-ranking male, the other by a low-ranking male. Seasonal effects were equally distributed over the two treatments. All birds were fed *ad libitum* during the experiment on a mix of wheat grain and high protein crumb with calcium and mineral supplements.

Eggs were incubated artificially under standardized conditions. Immediately on hatching, chicks were ringed with individual combinations and their morphometrics were recorded. All chicks were measured and weighed at weekly intervals until reaching adult size. Male moult development was scored weekly and males were assessed for attractiveness the following spring in the same manner as their fathers.

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Macaque monkeys categorize images by their ordinal number

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The recall of a list of items in a serial order is a basic cognitive skill¹. However, it is unknown whether a list of arbitrary items is remembered by associations between sequential items^{2,3} or by associations between each item and its ordinal position⁴. Here, to study the nonverbal strategies used for such memory tasks^{5–9}, we trained three macaque monkeys on a delayed sequence recall task. Thirty abstract images, divided into ten triplets, were presented repeatedly in fixed temporal order. On each trial the monkeys viewed three sequentially presented sample stimuli, followed by a test stimulus consisting of the same three images and a distractor image (chosen randomly from the remaining 27). The task was to touch the three images in their original order without touching the distractor. The most common error was touching the distractor when it had the same ordinal number (in its own triplet) as the correct image. Thus, the monkeys' natural tendency was to categorize images by their ordinal number. Additional, secondary strategies were used eventually to avoid the distractor images. These included memory of the sample images (working memory) and associations between sequence triplet members. Thus, monkeys use multiple mnemonic strategies according to their innate tendencies and the requirements of the task.

A basic question in the study of serial memory concerns the nature of the mental representation that allows retrieval of list items. Is list memory better characterized as a set of associations between adjacent items ('chaining'²) or even nonadjacent items³, or as a stored pattern of symbolic associations between each item and its ordinal position⁴? Humans can encode list items as words ('naming'), acquiring associations among names or between names and ordinal positions, and are very experienced with sequence tasks (numbers, alphabet, musical scale) and the abstract categories 'first', 'second' and so on. However, monkeys and even pigeons can reproduce ordered lists of arbitrary stimuli^{1,5–7,10–15}. What preverbal mnemonic strategies are used to represent lists?

In principle, multi-image working memory could be used to recall the identity of a stimulus and its temporal position in a sequence. This strategy is advantageous for generalization: it is as successful with new as with familiar stimuli, and is also successful with randomly ordered images. Monkeys can report whether a probe item was included in a previously presented list of up to 20 items almost as accurately as humans¹⁶. However, remembering the temporal position of an image is much more demanding. When monkeys were asked to choose which of two images appeared earlier in a list of five arbitrarily chosen items, their average accuracy was only ~ 73% (ref. 17).

When images are presented in fixed temporal order, subjects can use long-term memory strategies instead of working memory. One strategy is to generate an association between adjacent images in the

fixed sequence. Monkeys are highly skilled at generating such paired associations^{18–20}. Using a series of pair associations, a chain of images can be recalled. In fact, monkeys trained on a series of images successfully report the order of a random pair from the fixed sequence, even using non-sequential pairs^{5–7}. Similarly, monkeys trained on a sequence of pairs (A–B, B–C and so on) successfully report the order of any pair^{8,9}. In both cases analysis of response times indicates that they may use an internal serial recap of the list, perhaps on the basis of serial pair associations^{7,8,14}.

Another possible strategy is to memorize the ordinal position of each image. In one study, monkeys were trained on four nonverbal lists, each containing four novel photographs of natural objects, using the successive-phase method¹⁵. The task was to touch the simultaneously presented images in the correct order (A1–A2–A3–A4, B1–B2–B3–B4, C1–C2–C3–C4, D1–D2–D3–D4). When the monkeys had mastered this task, the items were shuffled, taking one item from each list, so that in two derived lists the ordinal number of the items was maintained (for example, A1–D2–C3–B4) whereas in two others it was not (for example, B3–A1–D4–C2). Lists with maintained ordinal position were acquired rapidly and virtually without error, whereas derived lists in which the ordinal position was changed were as difficult to learn as new lists. This pattern of transfer to derived lists implies that the monkeys originally acquired some knowledge about each item's ordinal position, rather than simply generating a chain of serial pair associations for each list of items.

Here we created an experimental paradigm in which monkeys could use all three of these strategies. This allowed us to assess which strategy is the most natural, being the first to be learned, and which is dominant, having the biggest effect on performance. The monkeys were trained on a task requiring them to report a list of three images in the order of their previous presentation. We used thirty fractal images, presented in fixed temporal order. These were divided into ten constant non-overlapping triplets (Fig. 1a). Each trial consisted of a triplet of three sample images, with each image

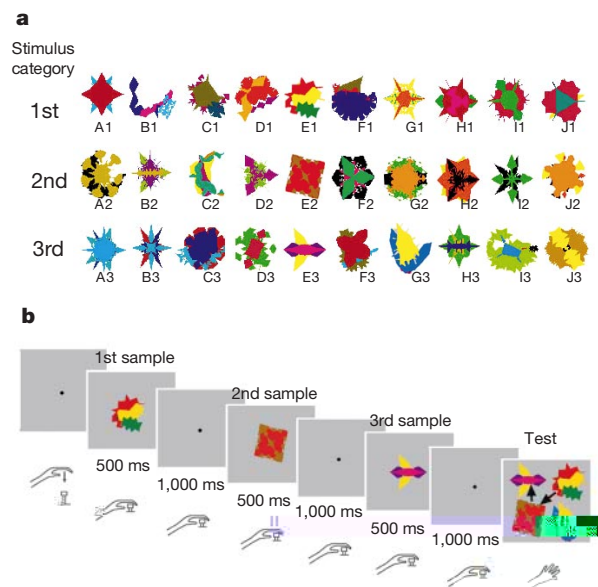


Figure 1 Image set and the basic delayed sequence recall task. **a**, A set of 30 fractal images were presented in a fixed temporal order using 10 constant non-overlapping triplets (columns). Each image has a fixed ordinal position in its triplet (1st, 2nd and 3rd) and the triplets were presented in a fixed order. The images were divided into three different categories (rows) according to their fixed ordinal positions in the triplets. **b**, Example of the sequence of events in a trial. Arrows at the 'Test' period mark the correct touching order.