Dynamic Paternity Allocation as a Function of Male Plumage Color in Barn Swallows

R. J. Safran,[1,2]* C. R. Neuman,[2] K. J. McGraw,[3] I. J. Lovette[1,2]

Paternity in male animals can be influenced by their phenotypic signals of quality. Accordingly, the behavior underlying patterns of paternity should be flexible as signals of quality change. To evaluate the dynamics of paternity allocation, we analyzed paternity before and after manipulating plumage coloration, a known signal of quality, in male barn swallows *Hirundo rustica*. We found that, in successive breeding bouts, only males whose plumage color was experimentally enhanced received greater paternity from their social mates, demonstrating evidence for flexible and dynamic paternity allocation and the importance for males of maintaining signals of quality well after pair bond formation.

Extrapair fertilizations are common in organisms with socially monogamous breeding systems (*1*, *2*). It is widely viewed that extrapair mating is an adaptive, flexible response to variability in the quality of potential mates within and among breeding attempts (*2*–*6*). However, despite dozens of studies on extrapair mating, we know remarkably little about the dynamics of paternity allocation. Many studies have shown differential allocation of paternity in relation to features of mate quality (*1*), but the strongest evidence for an association between male quality and paternity allocation would come from studies in which paternity was assessed both before and after male signals of quality are manipulated experimentally. However, to date, male ornament manipulations have been conducted only before (*7*, *8*) or just after (*9*–*11*) the male has formed a social pair bond and, in every case, before a first breeding attempt.

Comparing a male's paternity in successive breeding attempts, before and after his phenotype is manipulated, is critical for rigorously studying the dynamics of paternity allocation, because it allows one to (i) assess the dynamics of paternity allocation within the same breeding pair; (ii) control for potentially confounding variables such as female quality, familiarity between social mates, and interactions between female and male quality, all of which could strongly influence paternity allocation (*1*); and thereby (iii) analyze directly the relationship between successive paternity outcomes and whether they are affected by phenotypic signals of male quality.

[1]Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853, USA. [2]Evolutionary Biology Program, Cornell Laboratory of Ornithology, 159 Sapsucker Woods Road, Ithaca, NY 14850, USA. [3]School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA.

*Present address: Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544, USA. To whom correspondence should be addressed. E-mail: rsafran@princeton.edu

We studied the dynamics of paternity allocation as a function of an experimentally manipulated signal of mate quality in barn swallows (*Hirundo rustica erythrogaster*) from North America. Extrapair fertilizations are common in this socially monogamous species (*7*, *12*). Unlike in European populations of barn swallows (*H. r. rustica*), where elongate tail streamers function as sexual signals (*7*), ventral plumage coloration is a sexually selected trait in our study population of *H. r. erythrogaster* (*13*).

We used a paired design to test whether within-season changes in male coloration affect paternity allocation in two successive breeding attempts. Before the start of the experiment, we captured each adult and collected morphological data and a blood and feather sample (*14*). To determine whether individuals (females or conspecific males) assess male quality dynamically during the breeding season, we (i) allowed a female to settle with a mate and lay a complete clutch of eggs and (ii) recaptured and randomly assigned males to one of three treatment groups: their feather coloration was enhanced within the natural range of variation (fig. S1), or they were placed in one of two control groups, a sham manipulated group or an unmanipulated group (*15*). We simultaneously (iii) removed the first clutch to simulate a nest failure, thereby inducing the female to lay a replacement clutch after she had the opportunity to reassess her social mate's quality.

DNA samples from each embryo in the first clutch and from each nestling in the replacement brood were used to compare paternity allocation to the same male as a function of changes in signals of male quality by directly analyzing differences in the proportion and number of extrapair young between the first and the replacement clutches of males in each treatment group. We used microsatellite-based analyses to determine the paternity of offspring in first versus replacement broods in order to directly examine changes in a male's paternity in response to the experimental manipulation (*15*).

In the clutches laid before plumage color was manipulated, there were no initial differences in paternity across treatments [number of young sired by focal male/total number of young in clutch, logistic model, $\chi^2 = 0.09$, $P > 0.95$; number of a male's own young in nest, analysis of variance (ANOVA) $F_{2,\,27} = 0.07$, $P > 0.90$] (Fig. 1). In the subsequent breeding attempt, however, there was a significant effect of our plumage manipulation on paternity (differences in proportion of paternity, ANOVA $F_{2,\,24} = 4.0$,

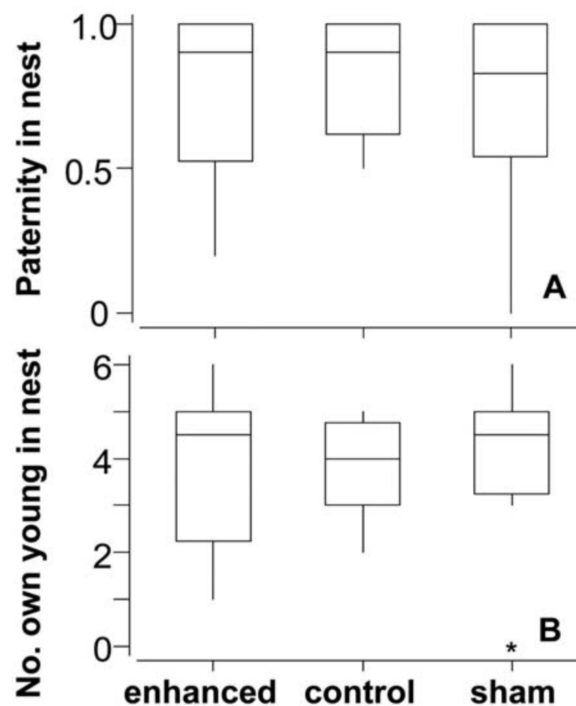

Fig. 1. (**A**) The proportion of within-pair young sired by males in three treatment groups did not differ at the start of the experiment. (**B**) The number of offspring in the nest that were sired by the focal male did not differ across treatments at the start of the experiment. These box and whisker plots portray the median value (line across box), and the first and third quartiles (boxes below and above median line, respectively). Whiskers indicate lines that extend from the bottom and top of the box to the lowest and highest values adjacent to the box that are defined by the following limits: lower limit = [quartile 1 − 1.5(quartile 3 − quartile 1)] and upper limit = [quartile 3 + 1.5(quartile 3 − quartile 1)]. The asterisk in (B) indicates an outlier outside of the lower limit.

$P < 0.04$; differences in number of a male's own young in nest, ANOVA $F_{2,\,23} = 5.45$, $P < 0.02$) (Fig. 2). Posthoc pairwise comparisons (Tukey's test at $P < 0.05$) indicate that the paternity and number of young of males in the enhanced treatment were significantly greater than the paternity or number of young of males in both control groups and that both control groups did not differ from one another for either measure of paternity. That males with enhanced plumage color gained paternity in replacement clutches, whereas males in both control groups did not, provides compelling evidence for a causal relationship between paternity and feather coloration and demonstrates that paternity allocation is dynamic between successive breeding attempts in this population of barn swallows.

Other nonexperimental studies have also reported differences in extrapair paternity rates between breeding bouts (*16*), providing further support for our finding that individuals rapidly adjust paternity in relation to mate quality. For example, male savannah sparrows (*Passerculus sandwichensis*) that provide high-quality parental care in a first brood receive greater paternity from their social mates in the subsequent breeding attempt (*17*).

Successive breeding bouts within the same pair bond and asynchronous breeding dates among breeding pairs are common in many socially monogamous species, suggesting that there should be a premium on the maintenance of ornamental traits even after pair bonds are formed. Indeed, the quality of ornaments including feather coloration (*18*, *19*) often declines within a breeding season, and other kinds of ornaments such as antlers or elongated plumes are subject to breakage and deterioration. Whether some males are better at maintaining their ornaments throughout a breeding season remains largely unknown.

Although the precise mechanism of paternity allocation was not tested in this study, mate choice and intrasexual competition may both have affected paternity outcomes (*2*, *20*–*22*). Females may exhibit some control over extrapair partner choice and fertilizations (*23*), and in European barn swallows there is experimental evidence for female choice of extrapair males with the longest tail streamers (*7*). However, it is also possible that males in our study with experimentally enhanced plumage prevented their mates from copulating with other males in the population. Melanin-based plumage color, like that exhibited by barn swallows (*24*), is used in other animals as an honest signal of dominance (*25*, *26*). Moreover, it is possible that both female choice and male-male competition favor the use of plumage color as a quality indicator in barn swallows, as has been discovered in recent experiments of melanin-based coloration in common yellowthroats, *Geothlypis trichas* (*27*). Lastly, it is possible that misrecognition of one's previous mate may have influenced the outcome of our experiment. However, sexually selected coloration in barn swallows (*13*) does not possess characteristics of traits typically used as signals of individual identity, such as discrete color morphs that do not signal reproductive performance (*28*). Additionally, many other characteristics of each male that could signal identity (e.g., song) were not manipulated in our experiment.

Whether the underlying mechanism is governed by female choice, male-male competition, or both, the allocation of greater paternity to males with experimentally enhanced plumage color, despite the fact that all females remained paired with their original social males, is consistent with the hypothesis that flexibility in paternity allocation is a direct response to changes in male coloration, indicating that individuals use this signal to gauge important aspects of a male's quality. Although there are no previous demonstrations of dynamic paternity allocation decisions in relation to male ornaments, it is easy to posit strong selection on the flexibility of these decision rules because the pursuit of extrapair matings by both males and females has been shown to have important fitness outcomes (*2*–*6*). Moreover, dynamic decision rules are evident within the context of male paternal care and paternity certainty in species where extrapair matings are prevalent (*29*, *30*), suggesting that flexibility and dynamic assessment in allocation decision rules is an important component of variable reproductive strategies.

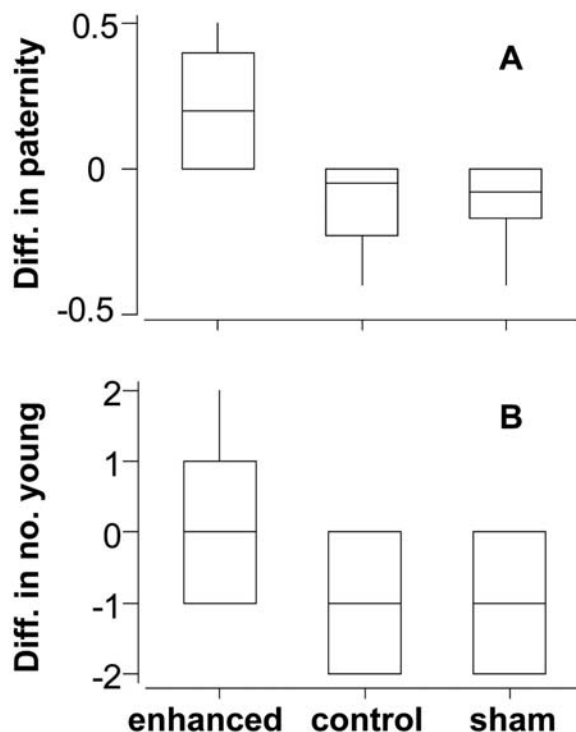

Fig. 2. (**A**) Differences in paternity for the replacement broods minus paternity for the first breeding attempts demonstrate that males whose coloration was enhanced gained more paternity, whereas males in both control groups lost or received no changes in paternity of young within their own nest. (**B**) Differences in the number of young sired by the focal male indicate that only males whose coloration was enhanced had greater numbers of their own offspring in replacement clutches, whereas males in both control groups had reduced numbers of their own offspring in replacement clutches. In these box and whiskers plots, whiskers indicate lines that extend from the bottom and top of the box to the lowest and highest values adjacent to the box that are defined by the following limits: lower limit = [quartile 1 − 1.5(quartile 3 − quartile 1)] and upper limit = [quartile 3 + 1.5(quartile 3 − quartile 1)].

References and Notes

1. S. C. Griffith, I. P. F. Owens, K. A. Thuman, *Mol. Ecol.* **11**, 2195 (2002).
2. D. F. Westneat, I. R. K. Stewart, *Annu. Rev. Ecol. Evol. Syst.* **34**, 365 (2003).
3. B. D. Neff, T. E. Pitcher, *Mol. Ecol.* **14**, 9 (2005).
4. H. L. Mays Jr., G. E. Hill, *Trends Ecol. Evol.* **19**, 554 (2004).
5. M. D. Jennions, M. Petrie, *Biol. Rev.* **75**, 21 (2000).
6. B. Kempenaers *et al.*, *Nature* **357**, 494 (1992).
7. N. Saino, C. R. Primmer, H. Ellegren, A. P. Møller, *Evolution* **51**, 562 (1997).
8. A. Johnsen, S. Andersson, J. Ornborg, J. T. Lifjeld, *Proc. R. Soc. London Ser. B* **265**, 1313 (1998).
9. H. Ellegren, J. T. Lifjeld, T. Slagsvold, C. R. Primmer, *Mol. Biol.* **4**, 739 (1995).
10. K. A. Otter *et al.*, *J. Avian Biol.* **32**, 338 (2001).
11. D. J. Mennill, L. M. Ratcliffe, P. T. Boag, *Science* **296**, 873 (2002).
12. H. G. Smith, R. Montgomerie, T. Põldmaa, B. N. White, P. T. Boag, *Behav. Ecol.* **2**, 90 (1991).
13. R. J. Safran, K. J. McGraw, *Behav. Ecol.* **15**, 455 (2004).
14. We studied a population of barn swallows at 15 breeding sites in Tompkins County, New York (42°30′N, 76°28′W), from May through August 2002. Individuals were uniquely marked, and all nests at these sites were visited every other day.
15. Materials and methods are available as supporting material on *Science* Online.
16. K. F. Conrad, R. J. Robertson, P. T. Boag, *Auk* **115**, 497 (1998).
17. C. R. Freeman-Gallant, *Proc. R. Soc. London Ser. B* **263**, 157 (1996).
18. K. J. McGraw, G. E. Hill, *Can. J. Zool.* **82**, 734 (2004).
19. J. Örnborg, S. Andersson, S. C. Griffith, B. C. Sheldon, *Biol. J. Linn. Soc.* **76**, 237 (2002).
20. C. Darwin, *The Descent of Man and Selection in Relation to Sex* (Murray, London, 1871).
21. M. B. Andersson, *Sexual Selection* (Princeton Univ. Press, Princeton, NJ, 1994).
22. R. R. Snook, *Trends Ecol. Evol.* **20**, 46 (2005).
23. T. R. Birkhead, T. Pizzari, *Nat. Genet.* **3**, 262 (2002).
24. K. J. McGraw, R. J. Safran, M. R. Evans, K. Wakamatsu, *Behav. Ecol.* **15**, 889 (2004).
25. E. A. Tibbetts, J. Dale, *Nature* **432**, 218 (2004).
26. S. Rohwer, E. C. Rohwer, *Anim. Behav.* **26**, 1012 (1978).
27. S. A. Tarof, P. O. Dunn, L. A. Whittingham, *Proc. R. Soc. London Ser. B* **272**, 1121 (2005).
28. J. Dale, D. B. Lank, H. K. Reeve, *Am. Nat.* **158**, 75 (2001).
29. B. D. Neff, *Nature* **422**, 716 (2003).
30. B. C. Sheldon, H. Ellegren, *Proc. R. Soc. London Ser. B* **265**, 1737 (1998).

31. We thank J. Anderson, M. Byrne, L. Stenzler, and S. McRae

for assistance; K. Grace-Martin and F. Vermeylen for statistical support; and D. Ardia, B. Brennan, P. Brennan, J. Dale, S. Flaxman, H. Greene, M. Hauber, D. Hawley, P. Sherman, A. Sugden, D. Winkler, and three anonymous reviewers for comments on the manuscript. Financial assistance was provided by the American Association of University Women, the American Ornithologists' Union, the Animal Behavior Society, the Department of Ecology and Evolutionary Biology at Cornell University, NSF, the Purple Martin Conservation Association, Sigma Xi, the Wilson Ornithological Society, Cornell University's Presidential Research Scholar's Program, and the Environmental Protection Agency.

Supporting Online Material
www.sciencemag.org/cgi/content/full/309/5744/2210/DC1
Materials and Methods
Fig. S1
Table S1

20 May 2005; accepted 1 August 2005
10.1126/science.1115090

Transmembrane Protein GDE2 Induces Motor Neuron Differentiation in Vivo

Meenakshi Rao and Shanthini Sockanathan*

During neural development, coordinate regulation of cell-cycle exit and differentiation is essential for cell-fate specification, cell survival, and proper wiring of neuronal circuits. However, the molecules that direct these events remain poorly defined. In the developing spinal cord, the differentiation of motor neuron progenitors into postmitotic motor neurons is regulated by retinoid signaling. Here, we identify a retinoid-inducible gene, *GDE2* (glycerophosphodiester phosphodiesterase 2), encoding a six-transmembrane protein that is necessary and sufficient to drive spinal motor neuron differentiation in vivo. A single amino acid mutation in the extracellular catalytic domain abolishes protein function. This reveals a critical role for glycerophosphodiester metabolism in motor neuron differentiation.

During development of the nervous system, cell-cycle exit is coupled to cellular differentiation programs to ensure that correct numbers of neuronal subtypes are generated to construct functional neural circuits (*1*). This complex process involves the synchronized decrease in expression of progenitor determinants, the increase of cell-cycle inhibitors, and the implementation of defined cell-fate specification programs. The molecular mechanisms that coordinate and regulate these pathways remain unclear.

Spinal motor neuron generation in the chick requires the integration of three different extrinsic signals: sonic hedgehog, fibroblast growth factors, and retinoic acid (RA) (*2*, *3*). All three signaling pathways have been implicated in initial dorsal-ventral patterning of progenitor domains in the spinal cord (Fig. 1A). However, RA signaling is also necessary for the induction of oligodendrocyte transcription factor 2 (Olig2) in progenitors and their subsequent differentiation into postmitotic motor neurons (Fig. 1A) (*2*). When motor neuron progenitors differentiate, they decrease expression of Olig2 as they migrate out of the ventricular zone (VZ) and increase expression of postmitotic motor neuron markers such as islet1 and islet2 (Fig. 1A) (*4*). Olig2 has a pivotal role in motor neuron differentiation. It is required for the maintenance of a motor neuron progenitor state, and its down-regulation is essential for the implementation of neurogenic and motor neuron specification pathways (*5*, *6*).

Because the differentiation of motor neuron progenitors is dependent on retinoid signaling, we conducted a differential subtraction screen with cDNAs derived from ventral spinal cord explants grown in the presence or absence of retinol to identify genes involved in this process (Fig. 1B) (*7*). Probing reverse Northern blots with cDNAs from both sets of explants demonstrated that expression of clone 45.1 was increased about 50-fold in explants exposed to retinol compared with that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Fig. 1C). Furthermore, in situ hybridization analysis revealed that clone 45.1 was expressed within or directly adjacent to developing tissues that synthesize RA, such as the spinal cord, paraxial mesoderm, mesonephros, heart, lung, and eye (fig. S1) (*8*). Sequence analysis revealed that clone 45.1 is a chick gene (AY910750) encoding a predicted protein of 599 amino acids with 67% identity to the human predicted protein PP1665 and 66% identity to mouse glycerophosphodiester phosphodiesterase 2 (GDE2) (*9*, *10*) (fig. S2), suggesting clone 45.1 is the chick homolog of GDE2. These proteins all contain a glycerophosphodiester phosphodiesterase (GDPD) domain, known to be involved in glycerophosphodiester metabolism (*11*). Analysis of the Conserved Domain Database revealed that GDE2 is a member of a large, heterogeneous family of GDPD-containing proteins for which in vivo functions are largely unknown (*9*). GDE2 is a transmembrane protein, and epitope tagging studies demonstrated that the GDPD domain is extracellular with intracellular localization of the N- and C-termini (fig. S3).

GDE2 is highly expressed by all somatic spinal motor neurons, irrespective of their rostrocaudal position, from the time they are generated (Fig. 2, A to F) until at least Hamburger-Hamilton (HH) stage 29 (*8*). These data are consistent with the induction of GDE2 expression by paraxial mesoderm-derived RA signaling. In order to determine when GDE2 might act in motor neuron development, the onset of GDE2 expression was examined. The differentiation of motor neuron progenitors can be monitored accurately by the

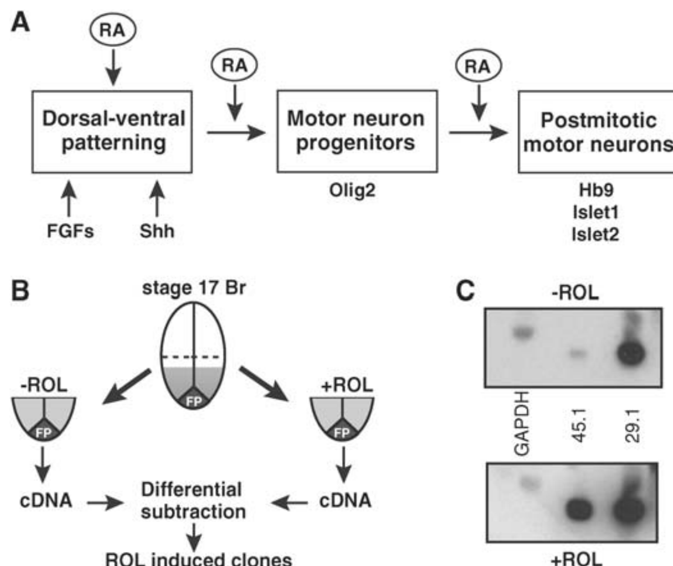

Fig. 1. GDE2 isolation and characterization. (**A**) Schematic depicting requirement for RA signaling at three distinct steps in motor neuron generation. Shh, Sonic hedgehog; FGFs, fibroblast growth factors. (**B**) Subtractive screen to isolate retinoid-responsive genes in motor neurons. Br, brachial neural tube; FP, floor plate; ROL, retinol. (**C**) Reverse Northern blots showing RA responsiveness of clone 45.1 when probed with cDNA from explants grown in the presence or absence of ROL compared with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and a non-RA-responsive clone, 29.1.

Department of Neuroscience, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, USA.

*To whom correspondence should be addressed. E-mail: ssockan1@jhmi.edu