29. C. M. Pease, R. Lande, J. J. Bull, *Ecology* **70**, 1657 (1989).
30. T. J. Case, M. L. Taper, *Am. Nat.* **155**, 583 (2000).
31. F. A. Smith, J. L. Betancourt, J. H. Brown, *Science* **270**, 2012 (1995).
32. E. A. Hadly, M. H. Kohn, J. A. Leonard, R. K. Wayne, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6893 (1998).
33. S. Chiba, *Paleobiology* **24**, 99 (1998).
34. J. Haffer, *Science* **165**, 131 (1969).
35. J. B. Losos, K. I. Warhelt, T. W. Schoener, *Nature* **386**, 70 (1997).
36. R. B. Huey, G.W. Gilchrist, M. L. Carlson, D. Berrigan, L. Serra, *Science* **287**, 308 (2000).
37. M. A. Bell, C. A. Andrews, in *Evolutionary Ecology of Freshwater Animals*, B. Streit, T. Städler, C. M. Lively, Eds. (Birkhäuser Verlag, Basel, Switzerland, 1997), pp. 323–363.
38. H. D. Rundle, L. Nagel, J. W. Boughman, D. Schluter, *Science* **287**, 306 (2000).
39. O. Folmer, M. Black, W. Hoeh, R. Lutz, R. Vrijenhoek, *Mol. Mar. Biol. Biotechnol.* **3**, 294 (1994).
40. D. L. Swofford, *PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods)*, version 4.062a (Sinauer, Sunderland, MA, 1998).
41. S. Schneider, D. Roessli, L. Excoffier, *Arlequin: A Software for Population Genetics Data Analysis*, version 2.000 (Genetics and Biometry Lab, Department of Anthropology, Univ. of Geneva, Switzerland, 2000).
42. K. Roy, M. Foote, *Trends Ecol. Evol.* **12**, 277 (1997).
43. M. Nei, *Molecular Evolutionary Genetics* (Columbia Univ. Press, New York, 1987).
44. F. Tajima, *Genetics* **105**, 437 (1983).
45. We thank C. Cunningham, D. Jablonski, J. R. Kohn, R. Lande, P. Marko, J. Neigel, M. Noor, T. D. Price, M. Taylor, J. W. Valentine, J. Wares, and two anonymous reviewers for comments and/or discussions; P. Arbour-Reily and N. Crochet for technical help; and J. H. McLean, L. T. Groves (Natural History Museum of Los Angeles County), and P. D. Roopnarine (California Academy of Sciences) for access to museum collections and specimen loans. Supported by NSF grants (K.R. and M.E.H.).

22 February 2001; accepted 25 April 2001

Parent-Offspring Coadaptation and the Dual Genetic Control of Maternal Care

Aneil F. Agrawal,* Edmund D. Brodie III, Jeremy Brown

In many animal species, the amount of care provided by parents is determined through a complex interaction of offspring signals and responses by parents to those signals. As predicted by honest signaling theory, we show that in the burrower bug, *Sehirus cinctus*, maternal provisioning responds to experimental manipulations of offspring condition. Despite this predicted environmental influence, we find evidence from two cross-foster experiments that variation in maternal care also stems from two distinct genetic sources: variation among offspring in their ability to elicit care and variation among parents in their response to offspring signals. Furthermore, as predicted by maternal-offspring coadaptation theory, offspring signaling is negatively genetically correlated with maternal provisioning.

Parent-offspring conflict occurs because the fitness of parents and the fitness of individual offspring are maximized at different levels of parental investment (*1*, *2*). A variety of models predict that the evolutionarily stable (ESS) level of parental care lies somewhere between the values that maximize parent and offspring fitness (*2*, *3*). Offspring influence the ESS because parents respond to signals produced by offspring. In most circumstances, parents are expected to respond to offspring signals, either active or passive, that reliably indicate condition (*4*–*7*). Differences among offspring in the signals they produce are typically thought to result from environmental influences on condition. The nonexclusive possibility that signaling differences among offspring are genetically based has been relatively unexplored (*8*).

The behavioral interaction between parents and offspring generates a complex form of inheritance wherein genes in two individuals can influence the phenotype of a single trait, parental care (*9*–*11*). Parent-offspring conflict theory has focused on evolutionary endpoints rather than processes primarily because of ". . .the immense difficulty in understanding the genetics underlying parent-offspring conflict" (*2*). Consequently, even the most fundamental genetic assumptions, such as the existence of genetic variance for both parental and offspring components, remain largely untested (*8*). Furthermore, important theoretical predictions cannot be evaluated without measuring other aspects of the genetic architecture of parental care. When selection favors an intermediate level of parental care, many combinations of parent and offspring genotypes are equally fit (*10*, *11*). This selection for maternal-offspring coadaptation is expected to generate a negative correlation between the genetic components expressed by parents and offspring (*12*).

Burrower bugs (*13*) (*Sehirus cinctus*, Hemiptera: Cydnidae) exhibit maternal care (*14*–*16*). Females lay clutches of approximately 40 to 150 eggs in shallow burrows in the soil. A female guards her clutch for about 10 days until the eggs hatch. At that time, she begins collecting small mint nutlets (*Lamium* spp.) that she deposits in the burrow to provision her offspring (Fig. 1). Provisioning is not directed to individual offspring, but rather to the clutch as a whole, and continues through the end of the second stadium (about 10 days after hatching). Care is obligate; unprovisioned clutches do not survive (*17*). Although specific offspring signals have not been identified, the influence of offspring on provisioning can be observed. Because a female can produce multiple clutches within a season, it is possible that maternal care expended on one clutch reduces a female's residual reproductive value (*18*).

Parental and offspring influences on provisioning are difficult to disentangle because of covariances that are expected to exist between parents and their offspring (*9*, *10*, *19*). Cross-fostering [Experiments I and II (*20*, *21*)] eliminates genetic and phenotypic sources of covariance between parents and the offspring for which they care (*22*, *23*). We cross-fostered clutches after egg deposition, so our design may not completely eliminate potential pre-hatching sources of covariance. By splitting clutches [Experiment II (*21*)], we could detect the effect of offspring signaling on maternal provisioning averaged over multiple unrelated maternal genotypes. Using these techniques, we show that burrower bug (i) females respond to offspring signals, (ii) females respond differently to offspring whose condition has been experimentally reduced, (iii) offspring vary genetically in their ability to elicit provisioning, and (iv) maternal and offspring genetic components of provisioning are negatively correlated.

If females respond to offspring signals and if signaling intensity increases with offspring number, then females rearing larger clutches should provision more than females rearing smaller clutches (*24*). However, a relation between clutch size and provisioning could exist simply because females that produce large clutches are also better providers. We tested these alternatives by evaluating the influence of biological and foster clutch size on provisioning in 138 cross-fostered mother-offspring pairs (*20*, *25*). Consistent with the offspring signaling hypothesis, foster clutch size had a significant effect on provisioning (t test: $t = 6.78$, df = 135, $P < 0.0001$), whereas biological clutch size did not ($t = -0.01$, df = 135).

We performed a separate experiment to determine if offspring condition influenced maternal provisioning. We manipulated offspring condition by removing provisioned

Department of Biology and Center for the Integrative Study of Animal Behavior, Indiana University, Bloomington, IN 47405–3700, USA.

*To whom correspondence should be addressed. E-mail: aagrawal@bio.indiana.edu

nutlets in a paired split-clutch design (*21*). Each clutch was divided in half, and each half-clutch was raised by a different unrelated foster mother. Half-clutches from the same family were placed in two treatments: one in which provisioned nutlets were removed from the half-clutch and one in which females were allowed to provision normally. This paired split-clutch design allows comparisons to be made across treatments while controlling for differences in both offspring number and genotype. If mothers use signals that reflect offspring condition, there should be differences in maternal provisioning between the two treatments. Indeed, mothers in the nutlet removal treatment provisioned significantly more nutlets than mothers in the control treatment (Fig. 2). Alternatively, these differences may result from mothers responding directly to experimental nutlet removal rather than to cues from offspring. Further analysis does not support this alternative, but rather provides more evidence for offspring signaling.

If either active or passive signals are produced by offspring, then a genetic component to variation in signaling may exist and related individuals should exhibit positively correlated levels of elicitation (*26*). The split-clutch design allows us to test whether relative differences among families in the amount of provisioning elicited are consistent across treatments. Specifically, the offspring signaling hypothesis predicts that genetic variation in offspring elicitation will generate a positive correlation between the number of nutlets provisioned to each family split across the two treatments. We found that such a correlation exists ($\rho = 0.42$, $P < 0.05$), controlling for differences in family size.

We did not measure offspring signals directly. Nonetheless, we have demonstrated that mothers not only respond to offspring, but that they respond differently to offspring of different genotypes. The effect of foster clutch size on provisioning suggests that mothers respond positively to increased signaling (*24*). Through experimental manipulation, we also found evidence that the signal used by mothers is an indicator of condition.

Maternal-offspring coadaptation theory predicts that offspring and maternal influences on care will be negatively genetically correlated (*12*). We tested this prediction by measuring the association between the amount of provisioning by a mother to a foster clutch and the number of nutlets elicited by her biological clutch from an unrelated foster mother (*19*, *27*). Because cross-fostering eliminates all post-hatching environmental sources of covariation, any correlation between mothers and their offspring should be due to genetic factors. We found a significant negative correlation between 129 cross-fostered pairs of mothers and offspring (Fig. 3; $\rho = -0.26$, $P < 0.005$). The actual genetic correlation between parent and offspring components of provisioning may be more than twice as large as the observed correlation for two reasons (*22*): the relatedness coefficient between mothers and offspring is 0.5 and environmental effects may contribute to the variance in parent and/or offspring components, but not to their covariance. Alternatively, prehatching environmental sources of covariance may render the genetic correlation weaker than the value observed. As long as such environmental sources of covariance do not both oppose and outweigh the genetic factors, the negative correlation suggests that mothers that are genetically predisposed to be good providers tend to produce offspring that are genetically predisposed to be weak elicitors, and vice versa.

Our data show that mothers are influenced by their offspring and that genetic variation is present for both maternal and offspring components. Although we do not provide direct evidence of genetic variation for maternal provisioning, the correlation between maternal provisioning and offspring elicitation implies its existence (*8*, *22*). Maternal care behavior is thereby influenced by genes expressed in two genomes. Variation among mothers in their genetic predisposition to provision is a direct source of genetic variation. Genetic variation in offspring signaling is an indirect source of genetic variation because mothers respond to signals from offspring. Furthermore, these sources of variation are not independent; maternal and offspring components cannot evolve independently because of the genetic correlation between the two traits. Such dual genetic control produces a complex web of genetic effects that can alter the evolutionary trajectories of parental

Fig. 1. A female burrower bug is shown in the nest with her offspring. Offspring are feeding on the small gray mint nutlets that she has provisioned. [Photograph, E. D. Brodie III]

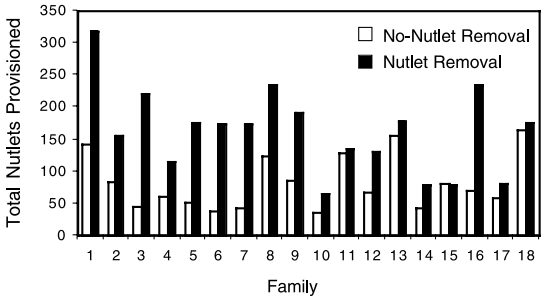

Fig. 2. Effects of nutlet removal on maternal provisioning. The total number of nutlets provisioned to half-clutches in the nutlet removal (solid) and no-nutlet removal (open) treatments is shown. Half-clutches with limited access to provisioned resources elicited significantly more maternal care than related half-clutches in a control treatment (Wilcoxon signed-rank test: $T_s = 84.5$, $P < 0.0001$).

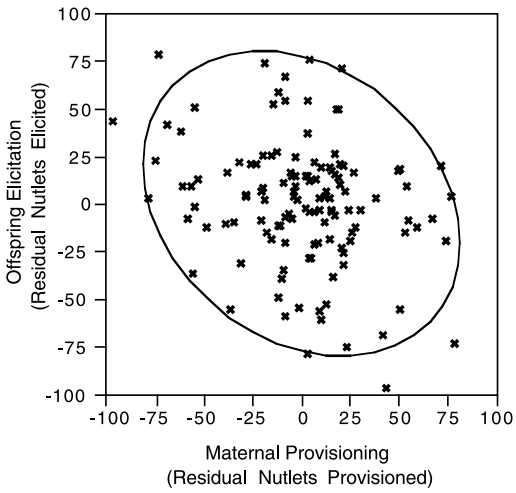

Fig. 3. Within-family relation of maternal provisioning and offspring elicitation. Number of nutlets elicited by offspring (from unrelated foster mothers) is plotted against number of nutlets provided by their biological mothers (to unrelated foster offspring). The 95% density ellipse is shown. These traits are significantly correlated ($\rho = -0.26$, $P < 0.005$) within families indicating a negative genetic correlation. Residual values are reported to control for variation in clutch size.

care and the offspring traits it influences in ways not predicted by traditional genetic models (*9*, *19*, *28*).

Much theoretical work has focused on the importance of honest signaling in social interactions (*2*, *4*, *6*, *29*). How do we interpret our results that signaling is affected by both condition and genotype in the context of honest signaling models? There are two possibilities. First, signal variation completely reflects differences in condition, but an individual's condition is influenced by both environmental and genetic factors. Even when all individuals experience similar environments, individuals will vary in condition because some individuals have better genotypes than others. Therefore, the genes that influence condition also affect signaling. The second possibility is that all genotypes signal more strongly with decreasing condition, but the absolute strength of signal produced for a given condition level varies among genotypes. In this case, signaling is only partially honest because it only partially reflects condition.

References and Notes

1. R. L. Trivers, *Am. Zool.* **14**, 249 (1974).
2. H. C. J. Godfray, *Nature* **376**, 133 (1995).
3. D. W. Mock, G. A. Parker, *The Evolution of Sibling Rivalry* (Oxford Univ. Press, New York, 1997).
4. A. Grafen, *J. Theor. Biol.* **144**, 517 (1990).
5. H. C. J. Godfray, *Nature* **352**, 328 (1991).
6. R. A. Johnstone, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 12644 (1999).
7. C. T. Bergstrom, M. Lachmann, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 5100 (1998).
8. M. Kölliker, M. W. G. Brinkhof, P. Heeb, P. S. Fitze, H. Richner, *Proc. R. Soc. London B* **267**, 2127 (2000).
9. J. M. Cheverud, A. J. Moore, in *Quantitative Genetic Studies of Behavioral Evolution*, C. R. B. Boake, Ed. (Univ. of Chicago Press, Chicago, 1994), pp. 67–100.
10. M. J. Wade, in *Maternal Effects as Adaptations*, T. Mousseau, C. Fox, Eds. (Oxford Univ. Press, New York, 1999), pp. 5–21.
11. J. B. Wolf, *Evolution* **54**, 1882 (2000).
12. _____, E. D. Brodie III, *Evolution* **52**, 299 (1998).
13. Female burrower bugs, *S. cinctus*, were collected from fields of *Lamium purpureum* in Bloomington, IN, in April 2000. Subjects were housed individually in 100 mm by 15 mm petri dishes with sand substrate and a plastic shelter until clutches were laid.
14. R. W. Sites, J. E. McPherson, *Ann. Entomol. Soc. Am.* **75**, 210 (1982).
15. S. L. Kight, *Anim. Behav.* **53**, 105 (1997).
16. _____, *Physiol. Entomol.* **23**, 38 (1998).
17. A. F. Agrawal, E. D Brodie III, J. Brown, unpublished data.
18. D. W. Tallamy, W. P. Brown, *Anim. Behav.* **57**, 727 (1999).
19. M. Kirkpatrick and R. Lande, *Evolution* **43**, 485 (1989).
20. Experiment I: Cross-fostering was achieved by placing each mother overnight in a small petri dish (60 mm by 15 mm) with an unrelated clutch 5 days after egg deposition. Under these conditions, adoption rate was high (>90%). Clutches and foster mothers were transferred into large petri dishes (150 mm by 15 mm) containing a plastic shelter. The number of offspring from each clutch was recorded upon hatching (~10 days after laying). Three bottle caps were distributed in the petri dish, and each cap was filled with 12 *L. purpureum* nutlets. The number of nutlets remaining in each dish was scored daily, and the total number of nutlets removed was recorded as a measure of provisioning. Each dish was restocked with 12 nutlets daily until the offspring reached third stadium. Mothers were frequently observed transporting nutlets from food dishes to the shelter, but offspring were never observed moving nutlets. Large numbers of nutlets were found in and around the shelter where offspring were usually clustered. Because burrower bugs have sucking mouthparts, adults do not need to remove nutlets from the food dish in order to consume their contents. Nutlets were rarely found in other areas of the experimental arena.
21. Experiment II: Immediately after hatching, 18 clutches were counted, split in half, and placed with an unrelated foster mother. Only clutches of sufficient size to generate half-clutches within the natural range of variation of full clutch size were used. Clutches and foster mothers were moved into experimental arenas as described above. For each pair of related half-clutches, one half-clutch was assigned to the "no-nutlet removal" (control) treatment and the other to the "nutlet removal" treatment. In each treatment, the nutlets remaining in each dish were counted and dishes were restocked daily every 2 hours from 7:00 a.m. to 9:00 p.m. until the offspring reached the third stadium. In the nutlet removal treatment, all nutlets provisioned to offspring were removed every 2 hours during counting. All nutlets provisioned were left with offspring in the control treatment. In the no-nutlet treatment, offspring had limited opportunity to feed on provisioned nutlets before they were removed.
22. M. Lynch, B. Walsh, *Genetics and Analysis of Quantitative Traits* (Sinauer, Sunderland, MA, 1998).
23. As a control for the cross-fostering, some individuals were "mock-fostered." In this treatment, mothers and egg clutches experienced the same type of manipulation as with cross-fostering, except that mothers re-adopted their own (i.e., related) egg clutch rather than an unrelated egg clutch. No differences existed in the total number of nutlets provisioned to 46 mock-fostered and 153 cross-fostered clutches (t = 0.892, df = 197). Neither did cross-fostering influence the size of offspring ($F_{1,193}$ = 2.15).
24. C. M. Rauter, A. J. Moore, *Proc. R. Soc. London B* **266**, 1691 (1999).
25. The influence of biological clutch size and foster clutch size on total nutlets provisioned was compared with the use of a multiple linear regression. We tested for effects of female body size (measured as pronotum width) in the same regression model, but no relation was apparent (t = 0.69, df = 127). Therefore, we report the results of the regression of total nutlets provisioned on biological clutch size and foster clutch size only.
26. We tested this one-tailed hypothesis using a product-moment correlation between the number of nutlets provisioned per individual offspring to a family in the two treatments. For statistical analyses, we used the residual from the regression of total nutlets provisioned on clutch size as a measure of nutlets per individual offspring. The results remain qualitatively similar if the analysis is performed with either the total nutlets provided or the ratio of nutlets/individual offspring.
27. The association between the number of nutlets a mother provisioned to her unrelated foster clutch and the number of nutlets elicited by her biological clutch from an unrelated foster mother was assayed with product-moment correlation. Differences in clutch size among families were adjusted for using residuals from the regression of total provisioning on foster clutch size.
28. A. J. Moore, E. D. Brodie III, J. B. Wolf, *Evolution* **51**, 1352 (1997).
29. R. Kilner, R. A. Johnstone, *Trends Ecol. Evol.* **12**, 11 (1997).

30. We thank K. Quinlan for assistance with bug collection and husbandry. M. Maple and M. Wade provided helpful comments on the manuscript. This work was supported by NSF grant IBN-9896116 to E.D.B. III and a Natural Sciences and Engineering Research Council of Canada Fellowship to A.F.A.

15 February 2001; accepted 24 April 2001

Sorting of Mannose 6-Phosphate Receptors Mediated by the GGAs

Rosa Puertollano,[1]* Rubén C. Aguilar,[1]* Inna Gorshkova,[2] Robert J. Crouch,[2] Juan S. Bonifacino[1]†

The delivery of soluble hydrolases to lysosomes is mediated by the cation-independent and cation-dependent mannose 6-phosphate receptors. The cytosolic tails of both receptors contain acidic-cluster-dileucine signals that direct sorting from the trans-Golgi network to the endosomal-lysosomal system. We found that these signals bind to the VHS domain of the Golgi-localized, γ-ear–containing, ARF-binding proteins (GGAs). The receptors and the GGAs left the trans-Golgi network on the same tubulo-vesicular carriers. A dominant-negative GGA mutant blocked exit of the receptors from the trans-Golgi network. Thus, the GGAs appear to mediate sorting of the mannose 6-phosphate receptors at the trans-Golgi network.

Lysosomal hydrolases are posttranslationally modified in the Golgi complex by the addition of mannose 6-phosphate groups that function as signals for sorting to lysosomes (*1*). The mannose 6-phosphate groups are recognized in the trans-Golgi network (TGN) by a cation-independent mannose 6-phosphate receptor (CI-MPR) or a cation-dependent mannose 6-phosphate receptor (CD-MPR). Both mannose 6-phosphate receptors (MPRs) mediate recruitment of the lysosomal hydrolases to clathrin-coated areas of the TGN, from which carrier vesicles deliver the MPR-hydrolase complexes to endosomes. The acidic pH of endosomes induces release

[1]Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA. [2]Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: juan@helix.nih.gov