

- (electron spin resonance) in the Mousterian cave deposits at Divje Babe I, Slovenia. *Geochaeology* 12, 507–536 (1997).
11. Brown, H. M. in *The New Grove's Dictionary of Music and Musicians* (ed. Sadie, S.) 664–681 (Macmillan, London, 1987).
 12. Pian, R. C., Kishibe, S. & Yang, B. N. in *The New Grove's Dictionary of Music and Musicians* (ed. Sadie, S.) 245–283 (Macmillan, London, 1987).
 13. Needham, N. J. T. M., Wang, L. & Robinson, K. G. in *Science and Civilization in China* iv/1 (ed. Needham, N. J. T. M.) 126–228 (Cambridge University Press, 1962).
 14. Lindley, M. in *The New Grove's Dictionary of Music and Musicians* (ed. Sadie, S.) 277–279 (Macmillan, London, 1987).

Supplementary Information is available at Nature's World-Wide Web site (<http://www.nature.com>) or as paper copy from the London editorial office of Nature.

Acknowledgements

This project was supported by the National Natural Science Foundation of China. In addition, C.W. was supported by the Department of Science & Technology of China, the Chinese Academy of Sciences and the Structure Research Laboratory at USTC. Research at Brookhaven National Laboratory is supported by the US Department of Energy. We thank Huang Xiangpeng of the Music School of the Art Institute of China who supervised these important tests and the personnel of the same Music School who carried them out: Xiao Xinghua, Xu Taoying, Gu Bobao, Tong Zhongliang, Qiu Ping and Liu Haiwang.

Correspondence should be addressed to G.H. (e-mail: garman@bnl.gov) and requests for materials should be addressed to J.Z. For further information see http://www.pubaf.bnl.gov/pr/news_releases.html

Incorporating rules for responding into evolutionary games

John M. McNamara*, Catherine E. Gasson† & Alasdair I Houston†

* School of Mathematics, University of Bristol, University Walk, Bristol BS8 1TW, UK

† School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, UK

Evolutionary game theory^{1,2} is concerned with the evolutionarily stable outcomes of the process of natural selection. The theory is especially relevant when the fitness of an organism depends on the behaviour of other members of its population. Here we focus on the interaction between two organisms that have a conflict of interest. The standard approach to such two-player games is to assume that each player chooses a single action and that the evolutionarily stable action of each player is the best given the action of its opponent. We argue that, instead, most two-player games should be modelled as involving a series of interactions in which opponents negotiate the final outcome. Thus we should be concerned with evolutionarily stable negotiation rules rather than evolutionarily stable actions. The evolutionarily stable negotiation rule of each player is the best rule given the rule of its opponent. As we show, the action chosen as a result of the negotiation is not the best action given the action of the opponent. This conclusion necessitates a fundamental change in the way that evolutionary games are modelled.

Most two-player game models with a continuous range of possible actions assume that: (1) each player makes its choice before it has observed the action of its opponent; (2) a player cannot change its action once the opponent's action has been observed; (3) all population members that have a given role in the game are identical. For example, if each member of a pair of animals chooses the proportion of time it spends scanning for predators as opposed to feeding, then each would prefer to feed as much as possible and so would prefer the other to be vigilant. In models of this conflict^{3–5}, all the above assumptions hold; in particular, assumption (3) holds because the models assume (sometimes implicitly) that all animals are identical in terms of both energy reserves and ability to detect and escape from predators. If popula-

tion members follow an evolutionarily stable strategy, assumption (3) implies that every player in a given role adopts the same action, so that each player effectively knows the action of its opponent before this action is observed. Thus, given assumption (3), assumption (2) is reasonable. It is, however, not reasonable to assume that players in a given role are identical. Individuals will differ in aspects of quality that influence the costs and benefits of taking an action, and hence the action chosen will depend on quality. If the quality of an opponent cannot be directly observed, then an opponent's action is not known in advance and it will be beneficial to respond to this action once it has been observed. The opponent should respond in turn, and so on, until both players reach a negotiated settlement.

It might be thought that, if the variation in quality is small, this variation, and hence negotiation, can be ignored. We show, however, that this approximation may give quantitatively different predictions even when the variation in quality is vanishingly small.

We present an analysis of negotiation in the context of a pair of animals feeding their young, but the qualitative conclusions apply to a wide class of games. If a parent increases its feeding effort this will reduce its own future reproductive success, but will increase the success of itself and its partner in the current breeding attempt. Thus a conflict of interest exists, with each parent preferring the other to work hard. There is evidence that a parent responds directly to the effort of its partner^{6,7}, so that a model involving negotiation seems appropriate. In contrast, the standard model of parental effort by Houston and Davies⁸ involves a single decision. This game can be summarized as follows. All members of a given sex are identical. Each parent makes a single choice of effort, ignoring the partner's effort, so that there is no negotiation. If the male provides effort u_m and the female provides effort u_f then $B(u_m + u_f)$ young survive to maturity. These efforts reduce the future reproductive success of the male and the female by $K_m(u_m)$ and $K_f(u_f)$, respectively. For a given female effort u_f , the best male effort $\hat{r}_m(u_f)$, is the value of u_m that maximizes

$$B(u_m + u_f) - K_m(u_m) \tag{1}$$

The best female effort given that of the male, $\hat{r}_f(u_m)$, is defined

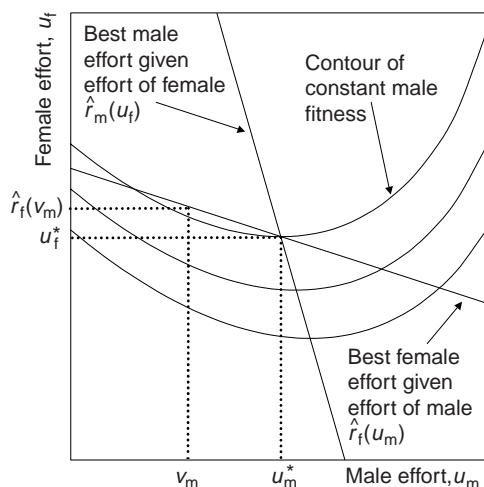


Figure 1 The best effort of a male, $\hat{r}_m(u_f)$, given fixed female effort u_f , and the best effort for the female, $\hat{r}_f(u_m)$ given fixed male effort u_m . The evolutionarily stable levels of effort, u_m^* and u_f^* , are the coordinates of the intersection of these two functions. The contours are lines of constant fitness for the male. For a given male effort, fitness increases with female effort. Assume that the female responds to the male with response rule \hat{r}_f . Then if a male provides effort v_m , the female responds with $\hat{r}_f(v_m)$ and the point $(v_m, \hat{r}_f(v_m))$ is above the contour that passes through (u_m^*, u_f^*) . Thus the male's fitness is higher than if he had used fixed effort u_m^* or had used \hat{r}_m as a response rule.

analogously. The evolutionarily stable male effort, u_m^* , and female effort, u_f^* , satisfy

$$u_m^* = \hat{r}_m(u_f^*) \quad \text{and} \quad u_f^* = \hat{r}_f(u_m^*) \quad (2)$$

Thus, the effort of every male is best given the effort of its partner and vice versa. The functions \hat{r}_m and \hat{r}_f and the resulting evolutionarily stable levels of effort are shown in Fig. 1.

The functions \hat{r}_m and \hat{r}_f specify the response over evolutionary time of one sex to the fixed effort of the other sex. The functions have been interpreted as specifying how one pair member should behaviourally respond to the observed effort of its partner⁶⁻¹¹. This interpretation is incorrect, as then the population would not be evolutionarily stable. To see this, suppose that all population members adopted these behavioural response rules. The male and female members of a pair would then alternate their responses until their efforts u_m^* and u_f^* satisfied equation (2). The total reproductive success of a single mutant male that uses fixed effort u_m would be

$$B(u_m + \hat{r}_f(u_m)) - K_m(u_m) \quad (3)$$

This male can exploit the response rule of the female by taking his effort to be just less than u_m^* , as the female will then partially compensate for his lack of effort by increasing her own effort above u_f^* . Figure 1 shows that, as a consequence, the mutant male's reproductive success is greater than if he had used the response rule \hat{r}_m (or had used fixed effort u_m^*).

In contrast to the assumptions of the model of ref. 8, parents will differ in quality (for example, foraging ability), which will influence the rate at which they should feed their young. Thus, as parents have been observed to respond to each other^{6,7}, a realistic model must seek evolutionarily stable negotiation rules rather than evolutionarily stable levels of effort. It is not straightforward to find such rules in general, so we concentrate on the following special model.

A negotiation involves the members of the breeding pair alternating in their choice of effort in response to that of their partner until the efforts of both partners settle down to limiting values. We refer to these final efforts as the outcome of the negotiation. We assume that in this process the effort adopted by an individual of quality q in response to the effort u of its partner is a function $r(u, q)$ of u and q alone, and does not depend on efforts earlier on in the negotiation. We refer to the function $r(u, q)$ as the response rule of

the individual. If a male of quality q_m has response rule $r_m(u, q_m)$ and a female of quality q_f has rule $r_f(u, q_f)$, then the outcome of negotiation will be efforts \hat{u}_m and \hat{u}_f , respectively, where $\hat{u}_m = r_m(\hat{u}_f, q_m)$ and $\hat{u}_f = r_f(\hat{u}_m, q_f)$.

The negotiation phase is assumed to be cost free. If the outcome of negotiation is that a parent of quality q expends effort u , its loss in future reproductive success is $K(u, q)$, irrespective of its sex. An individual of high quality pays less of a cost for a given level of effort than an individual of low quality; specifically, $\frac{\partial^2 K}{\partial u \partial q} < 0$. By convention we set quality $q = 0$ to be that implicit in the model of ref. 8. Then q in our current model can be interpreted as a quality deviation. The evolutionarily stable efforts predicted by Houston & Davies⁸ are then given by $u_m^* = u_f^* = u^*$, where $B'(2u^*) = \frac{\partial K}{\partial u}(u^*, 0)$. If the quality deviations of both individuals are small, then it is reasonable that the final efforts are close to u^* . We can then Taylor-expand B to give the approximation

$$B(u_{\text{tot}}) = B(2u^*) + (u_{\text{tot}} - 2u^*)B'(2u^*) + \frac{1}{2}(u_{\text{tot}} - 2u^*)^2 B''(2u^*) \quad (4)$$

where $u_{\text{tot}} = u_m + u_f$. Our model takes this formula to be exact. By similar reasoning we take

$$K(u, q) = K + (u - u^*) \frac{\partial K}{\partial u} + q \frac{\partial K}{\partial q} + \frac{1}{2} \left[(u - u^*)^2 \frac{\partial^2 K}{\partial u^2} + 2q(u - u^*) \frac{\partial^2 K}{\partial q \partial u} + q^2 \frac{\partial^2 K}{\partial q^2} \right] \quad (5)$$

where K and its derivatives are evaluated at $u = u^*$ and $q = 0$.

As we show, the above form of B and K allows us to restrict attention to response rules of the form

$$r(u, q) = u^* + \rho + \mu q - \lambda(u - u^*) \quad (6)$$

where ρ , μ and λ are constants with $|\lambda| < 1$. Here ρ indicates bias from the baseline effort u^* and μ determines the dependence of an individual's effort on its own quality. The crucial parameter is the responsiveness λ , which measures the degree to which an individual's effort compensates for the lack of effort of its partner. The condition $|\lambda| < 1$ ensures that the successive efforts of a parent in the negotiation phase settle down to limiting values.

Suppose that almost all members of a population use a response rule of the form of equation (6) with the same constants ρ , μ and λ . Consider a single mutant individual that uses a negotiation rule that maximizes its reproductive success with every possible partner that it could have. We do not restrict the mutant to have a rule of the form of equation (6), and allow the mutant to base its efforts in the negotiation process on the whole history of the process before the present time. We show in Box 1 that the dependence of the final effort of this individual, $\tilde{r}(u, q)$, on its own quality and the final negotiated effort, u , of its partner is

$$\tilde{r}(u, q) = u^* + \tilde{\rho} + \tilde{\mu} q - \tilde{\lambda}(u - u^*) \quad (7)$$

where $\tilde{\rho} = f(\lambda)$, $\tilde{\mu} = g(\lambda)$ and $\tilde{\lambda} = h(\lambda)$ depend only on the responsiveness λ of population members. However, then the outcome of the negotiation is the same as if the mutant had just used $\tilde{r}(u, q)$ as a response rule in the negotiation, and the mutant can do no better than to adopt this simple response rule.

The function $\hat{r}_m = \hat{r}_f$ of Houston and Davies⁸ gives the best effort of an individual for a fixed effort of its partner. If we interpret this function as a negotiation rule (the HD rule) then the responsiveness under this rule is $\tilde{\lambda} = h(0)$ (as the partner's effort is fixed and hence has responsiveness $\lambda = 0$).

Within the class of response rules of the form of equation (6), the unique evolutionarily stable negotiation rule has responsiveness λ_{ESS} , which satisfies $\lambda_{\text{ESS}} = h(\lambda_{\text{ESS}})$. The other parameters are then given by $\mu_{\text{ESS}} = g(\lambda_{\text{ESS}})$ and $\rho_{\text{ESS}} = f(\lambda_{\text{ESS}})$. The stability of the outcome of negotiation is shown in Fig. 2. In Box 1 we show that

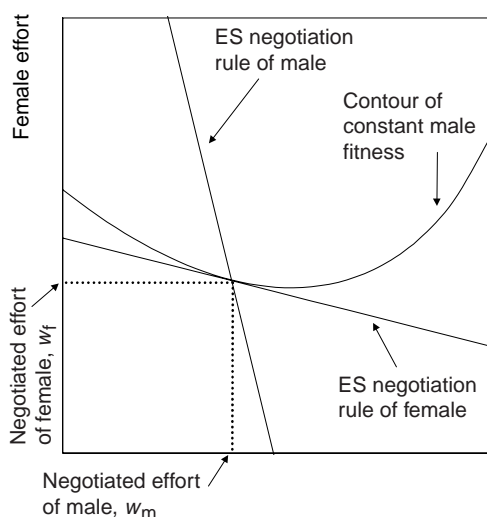


Figure 2 The evolutionarily stable negotiation rule for a male and its partner. If both players use this rule the resultant efforts will be w_m and w_f . The contour again shows constant fitness for the male. However, unlike in Fig. 1, this contour is now tangential to the evolutionarily stable negotiation rule of the female at (w_m, w_f) . Now any movement away from w_m will result in the male obtaining a lower fitness than if it had used the evolutionarily stable negotiation rule.

Box 1

Evolutionary stability

The evolutionarily stable (ES) effort of each parent, u^* , as predicted by the model of Houston and Davies⁸, is given by the solution to

$$\frac{\partial K}{\partial u}(u^*, 0) = B'(2u^*) \quad (8)$$

We assume that the marginal benefit to the brood decreases as parental effort increases. Thus B is a positive, increasing function and B'' is negative. Let $K_0 = \frac{\partial^2 K}{\partial u^2}(u^*, 0)$ and assume that the marginal cost of care increases as parental care increases, hence K_0 is positive. Let $K_1 = \frac{\partial^2 K}{\partial u \partial q}(u^*, 0)$, and assume that the marginal cost of care for a good quality individual is less than that for a poor quality individual, hence K_1 is negative.

The payoff to an individual of quality q providing effort u_1 whose partner provides effort u_2 is $R(u_1, u_2) = B(u_1 + u_2) - K(u_1, q)$. Taking the Taylor expansions (equations (4) and (5) to be exact and substituting from equation (8) we obtain $R(u_1, u_2) = (u_2 - u^*)B' + (u_1 + u_2 - 2u^*)^2 \frac{B''}{2} - (u_1 - u^*)^2 \frac{K_0}{2} - q(u_1 - u^*)K_1 - H(q)$, where $H(q)$ depends on q alone and B' and B'' are evaluated at $2u^*$.

We consider that females and males are identical and ignore any role asymmetries. We therefore refer to individuals' roles as player 1 or 2 rather than female or male. Consider a population in which an individual of quality q uses the response rule $r(u, q) = u^* + \rho + \mu q - \lambda(u - u^*)$, which is of the form of equation (6). We look for the optimal behaviour of a rare mutant within this population. Suppose that a rare mutant (player 1) of quality q_1 partners a resident population member (player 2) of quality q_2 . We allow the mutant to have full knowledge of its partner's quality and rule. We also allow the mutant to use any negotiating strategy providing that the negotiation eventually ends with well-defined efforts by both partners. If, after negotiation, player 1 provides effort u_1 , its payoff is

$$R(u_1, r(u_1, q_2)) = (r(u_1, q_2) - u^*)B' + (u_1 + r(u_1, q_2) - 2u^*)^2 \frac{B''}{2} - (u_1 - u^*)^2 \frac{K_0}{2} - q_1(u_1 - u^*)K_1 - H(q_1).$$

This is maximized when the final effort of player 1, \hat{u}_1 , satisfies $\frac{\partial R}{\partial u_1}(\hat{u}_1, r(\hat{u}_1, q_2)) = 0$, that is $0 = r_u(\hat{u}_1, q_2)B' + 2(\hat{u}_1 + r(\hat{u}_1, q_2) - 2u^*) \times (1 + r_u(\hat{u}_1, q_2)) \frac{B''}{2} - 2(\hat{u}_1 - u^*) \frac{K_0}{2} - q_1 K_1$, where r_u denotes the partial derivative of r with respect to effort u . As $r_u(u_1, q_2) = -\lambda$, the mutant can do no better than negotiate a final effort \hat{u}_1 satisfying

$$0 = -\lambda B' + 2(\hat{u}_1 + r(\hat{u}_1, q_2) - 2u^*)(1 - \lambda) \frac{B''}{2} - 2(\hat{u}_1 - u^*) \frac{K_0}{2} - q_1 K_1 \quad (9)$$

Given that player 1 negotiates effort \hat{u}_1 , the effort of player 2 after negotiation is $\hat{u}_2 = r(\hat{u}_1, q_1)$. Thus by equation (9), \hat{u}_1 and \hat{u}_2 are related by

$$0 = -\lambda B' + 2(\hat{u}_1 + \hat{u}_2 - 2u^*)(1 - \lambda) \frac{B''}{2} - (\hat{u}_1 - u^*)K_0 - q_1 K_1.$$

Rearranging this expression gives

$$\hat{u}_1 = u^* + \frac{-(\lambda B' + q_1 K_1 - (1 - \lambda)(\hat{u}_2 - u^*)B'')}{K_0 - (1 - \lambda)B''}.$$

Now consider a mutant rule under which the dependence of the negotiated effort of the mutant on its own quality q and its partner's negotiated effort u is given by

$$\tilde{r}(u, q) = u^* + \frac{-(\lambda B' + qK_1 - (1 - \lambda)(u - u^*)B'')}{K_0 - (1 - \lambda)B''} \quad (10)$$

Then the above analysis shows that, regardless of its quality, the mutant is doing the best it possibly can against every possible partner.

Given that a population uses the response rule $r(u, q) = u^* + \rho + \mu q - \lambda(u - u^*)$, a mutant that uses the response rule $\tilde{r}(u, q) = u^* + f(\lambda) + g(\lambda)q - h(\lambda)(u - u^*)$, where $f(\lambda) = \frac{-\lambda B'}{K_0 - (1 - \lambda)B''}$, $g(\lambda) = \frac{-K_1}{K_0 - (1 - \lambda)B''}$ and $h(\lambda) = \frac{-(1 - \lambda)B''}{K_0 - (1 - \lambda)B''}$ achieves the outcome of equation (10). Thus the optimal negotiation rule of a mutant is also a response rule of the form of equation (6).

From the above analysis there exists an ES negotiation rule within the class of response rules of the form of equation (6). This rule is given by $r_{ESS}(u, q) = u^* + \rho_{ESS} + \mu_{ESS}q - \lambda_{ESS}(u - u^*)$, where the responsiveness λ_{ESS} is a solution of the equation $\lambda_{ESS} = h(\lambda_{ESS})$, and the parameters μ_{ESS} and ρ_{ESS} are given by $\mu_{ESS} = g(\lambda_{ESS})$ and $\rho_{ESS} = f(\lambda_{ESS})$.

The responsiveness of the ES negotiation rule, λ_{ESS} , satisfies $\lambda_{ESS} = h(\lambda_{ESS})$ and thus is a solution of $B''\lambda_{ESS}^2 - (2B'' - K_0)\lambda_{ESS} + B'' = 0$. This equation has two roots; both are positive, only one is less than 1. We take λ_{ESS} to be the root satisfying $0 < \lambda_{ESS} < 1$. The parameter $\mu_{ESS} = g(\lambda_{ESS}) = \frac{-K_1}{K_0 - (1 - \lambda_{ESS})B''}$ is positive, as $K_0 > 0$, $K_1 < 0$, $B'' < 0$ and $1 - \lambda_{ESS} > 0$. The parameter $\rho_{ESS} = f(\lambda_{ESS}) = \frac{-\lambda_{ESS}B'}{K_0 - (1 - \lambda_{ESS})B''}$ is negative, as $B' > 0$ and $\lambda_{ESS} > 0$.

To show that the responsiveness of the ES negotiation rule is less than that of the HD rule we note that the function h is positive and decreasing and that λ_{ESS} is positive. Therefore $\lambda_{ESS} = h(\lambda_{ESS}) < h(0) = \hat{\lambda}$, the responsiveness of the HD rule.

The negotiated effort of a player of quality q_1 whose partner is quality q_2 is

$$u_{ESS}(q_1, q_2) = u^* + \frac{(1 - \lambda_{ESS})\rho_{ESS} + \mu_{ESS}(q_1 - \lambda_{ESS}q_2)}{(1 - \lambda_{ESS}^2)} \quad (11)$$

if both players use the ES negotiation rule, and $\hat{u}(q_1, q_2) = u^* + \frac{\hat{u}(q_1 - \lambda q_2)}{(1 - \lambda)}$ if both players use the HD rule.

It can be shown algebraically that so long as $q_1 + q_2$ is small enough that $q_1 + q_2 < \frac{(K_0 - 2B'')B'}{B''K_1}$, then $u_{ESS}(q_1, q_2) < \hat{u}(q_1, q_2)$. Hence, the negotiated effort using the ES negotiation rule is smaller than that using the HD rule.

The negotiated effort of a player is a strictly increasing function of its quality (for a given partner's quality), as differentiating equation (11) gives $\frac{\partial u_{ESS}(q_1, q_2)}{\partial q_1} = \frac{\mu_{ESS}}{(1 - \lambda_{ESS}^2)} > 0$.

$0 < \lambda_{ESS} < \hat{\lambda}$. Thus the evolutionarily stable negotiation rule is less responsive than the HD rule. We also show that, if any pair of individuals use the evolutionarily stable negotiation rule, the outcome is that each individual expends less effort than if the individuals used the HD rule. Thus, if individuals recognize each other's quality without negotiation, every member of every pair expends more effort than if quality is not directly observable and effort has to be negotiated. In particular, if both pair members have quality $q = 0$, then the final negotiated effort of both is less than u^* . Moreover, as the outcomes under the evolutionarily stable negotiation rule do not depend on the degree of quality variation (provided there is some variation), we do not even obtain the Houston and Davies efforts in the limit as quality variation tends to zero.

Our analysis generalizes to any game in which individuals can respond to each other's actions, and shows that the action chosen as a result of negotiation may not be the best given that of the opponent. For example, when two animals inspect a potential predator^{12,13}, after negotiation the distance of one animal from the predator may not be the best distance given the distance of the other animal. In many signalling games, the receiver responds to the signaller but the signaller cannot respond to the behaviour of the receiver¹⁴⁻¹⁷. A response by the signaller is, however, plausible in many situations. As an example, consider chicks using begging behaviour to signal need to a parent. If either foraging conditions for parents vary or all parents do not have the same foraging ability, then it will advantageous for a chick to alter its level of begging in response to the effort of its parent. This may result in negotiation

between the chick and the parent and the outcome may differ from that predicted by models in which there is no responding.

At evolutionary stability in our parental effort game, the negotiated effort of an individual's partner is a strictly increasing function of the partner's quality. Thus, after negotiation, an individual can infer the quality of its partner; that is, negotiated levels of effort are honest. Despite this, efforts are less than if individuals could directly observe quality. If population members behaved as if inferred quality had been directly observed, then a mutant individual that pretended to have lower quality in the negotiation phase would, after negotiation, expend low effort at the expense of its partner, who would compensate (Fig. 1). Thus, the population would not be evolutionarily stable. □

Received 2 March; accepted 1 July 1999.

1. Maynard Smith, J. *Evolution and the Theory of Games* (Cambridge Univ. Press, Cambridge, 1982).
2. Parker, G. A. & Maynard Smith, J. Optimality theory in evolutionary biology. *Nature* **348**, 27–33 (1990).
3. Pulliam, H. R., Pyke, G. H. & Caraco, T. The scanning behaviour of juncos: a game-theoretical approach. *J. Theor. Biol.* **95**, 89–103 (1982).
4. Lima, S. L. Vigilance while feeding and its relation to the risk of predation. *J. Theor. Biol.* **124**, 303–316 (1987).
5. McNamara, J. M. & Houston, A. I. Evolutionarily stable levels of vigilance as a function of group size. *Anim. Behav.* **43**, 641–658 (1992).
6. Wright, J. & Cuthill, I. C. Biparental care: short-term manipulation of partner contribution and brood size in the starling, *Sturnus vulgaris*. *Behav. Ecol.* **1**, 116–124 (1990).
7. Markman, S., Yom-Tov, Y. & Wright, J. Male parental care in the orange-tufted sunbird—behavioural adjustments in provisioning and nest guarding effort. *Anim. Behav.* **50**, 655–669 (1995).
8. Houston, A. I. & Davies, N. B. in *Behavioural Ecology: The Ecological Consequences of Adaptive Behaviour* (eds Sibly, R. M. & Smith, R. H.) 471–487 (Blackwell Scientific, Oxford, 1985).
9. Hatchwell, B. J. & Davies, N. B. Provisioning of nestlings by dunnocks, *Prunella modularis*, in pairs and trios: compensation reactions by males and females. *Behav. Ecol. Sociobiol.* **27**, 199–209 (1990).
10. Clutton-Brock, T. H. & Godfray, H. C. J. in *Behavioural Ecology* (eds Krebs, J. R. & Davies, N. B.) 234–262 (Blackwell Scientific, Oxford, 1991).
11. Mock, D. W. & Parker, G. A. *The Evolution of Sibling Rivalry* (Oxford Univ. Press, Oxford, 1997).
12. Dugatkin, L. A. *Cooperation Among Animals* (Oxford Univ. Press, Oxford, 1997).
13. Parker, G. A. & Milinski, M. Cooperation under predation risk: a data-based ESS analysis. *Proc. R. Soc. Lond. B* **264**, 1239–1247 (1997).
14. Grafen, A. Biological signals as handicaps. *J. Theor. Biol.* **144**, 517–546 (1990).
15. Maynard Smith, J. Honest signalling: the Philip Sidney game. *Anim. Behav.* **42**, 1034–1035 (1991).
16. Johnstone, R. A. Honest signalling, perceptual error and the evolution of 'all-or-nothing' displays. *Proc. R. Soc. Lond. B* **256**, 169–175 (1994).
17. Godfray, H. C. J. Signalling of need by offspring to their parents. *Nature* **352**, 328–330 (1995).

Acknowledgements

We thank I. Cuthill, S. Hunt, J. Hutchinson, I. Jewitt, P. Sozou and M. Yallop for comments on previous versions of this manuscript.

Correspondence and requests for materials should be addressed to J.M.M. (e-mail: john.mcnamara@bristol.ac.uk).

The liprin protein SYD-2 regulates the differentiation of presynaptic termini in *C. elegans*

Mei Zhen & Yishi Jin

Department of Biology, Sinsheimer Laboratories, University of California, Santa Cruz, California 95064, USA

At synaptic junctions, specialized subcellular structures occur in both pre- and postsynaptic cells. Most presynaptic termini contain electron-dense membrane structures¹, often referred to as active zones, which function in vesicle docking and release². The components of those active zones and how they are formed are largely unknown. We report here that a mutation in the *Caenorhabditis elegans* *syd-2* (for synapse-defective) gene causes a diffused localization of several presynaptic proteins and of a synaptic-vesicle membrane associated green fluorescent protein (GFP) marker^{3,4}. Ultrastructural analysis revealed that the active

zones of *syd-2* mutants were significantly lengthened, whereas the total number of vesicles per synapse and the number of vesicles at the prominent active zones were comparable to those in wild-type animals. Synaptic transmission is partially impaired in *syd-2* mutants. *syd-2* encodes a member of the liprin (for LAR-interacting protein) family of proteins which interact with LAR-type (for leukocyte common antigen related) receptor proteins with tyrosine phosphatase activity (RPTPs)^{5,6}. SYD-2 protein is localized at presynaptic termini independently of the presence of vesicles, and functions cell autonomously. We propose that SYD-2 regulates the differentiation of presynaptic termini in particular the formation of the active zone, by acting as an intracellular anchor for RPTP signalling at synaptic junctions.

We isolated the *syd-2(ju37)* mutant in a genetic screen for mutations affecting the localization of a GFP marker, *P_{unc-25}*-SNB-1::GFP, associated with the synaptic-vesicle membrane (refs 3, 4 and M.Z. and Y.J., unpublished). SNB-1 is a *C. elegans* synaptobrevin⁷, a synaptic-vesicle membrane protein involved in vesicle docking and exocytosis², and SNB-1::GFP is associated with vesicles³. The *unc-25* promoter drives the expression of SNB-1::GFP in the GABAergic DD and VD motorneurons^{4,8}. In wild-type animals carrying this marker, here called *juIs1*, GFP is seen as uniformly shaped fluorescent puncta evenly distributed along the ventral and dorsal nerve cords, which correspond to the presynaptic termini of 13 VD and 6 DD neurons, respectively⁹ (Fig. 1a, b). We found that, in *syd-2(ju37)* *juIs1* mutants, the total number of fluorescent puncta was the same as in wild-type animals (Fig. 1c–f). However, these puncta showed various degrees of diffusion, and the overall fluorescent intensity of GFP was reduced. In addition, three presynaptic proteins (synaptotagmin, syntaxin and RAB-3) were also diffusely localized in *syd-2* mutants (Fig. 1g, h; data not shown for syntaxin and RAB-3). *syd-2(ju37)* is a recessive mutation, and homozygous *syd-2(ju37)* animals showed mild defects in many visible behaviours, for example sluggish locomotion and defective egg laying. Immunocytochemistry and GFP markers revealed no abnormalities in the gross morphology of DD, VD and other motorneurons in *syd-2* mutants (data not shown). Thus, the diffuse SNB-1::GFP pattern in *syd-2(ju37)* mutants reflects a defect in presynaptic protein localization, but not in axonal guidance.

We cloned *syd-2* by germline transformation rescue (see Fig. 2 and Methods). The predicted SYD-2 protein is highly homologous to liprin- α (Fig. 2b)⁶. Liprin- α was first identified because it interacted with the second phosphatase domain of LAR-type RPTPs in the yeast two-hybrid assay⁵. In mammalian cells, liprin- α causes RPTPs to cluster to focal adhesions⁵. All liprins have coiled-coil domains at the amino terminal and three SAM domains at the carboxy terminal^{5,10}. SAM domains are protein modules that mediate homo- and heterodimerizations¹⁰. RPTPs interact with the C-terminal portion of liprin- α (ref. 5), and within this region SYD-2 and liprin- α 1 are 60% identical (Fig. 2b). In the yeast two-hybrid assay SYD-2 interacts with mammalian LAR and *Drosophila* DLAR (C. Serra-Pagès, M.Z., Y.J. and M. Streuli, unpublished results), indicating that SYD-2 is a functional homologue of liprin- α . Deletion of the three SAM domains (pCZ#10) greatly reduced *syd-2* rescuing ability (Fig. 2a), supporting the idea that the SAM domains are important for SYD-2 function. Moreover, the *ju37* mutation is probably a molecular null because it contained a C-to-T transition, changing glutamine 397 to an amber stop, and the predicted truncated protein was below the level of detection (Fig. 2b, c).

To gain insight into SYD-2 function, we analysed its expression pattern. Immunofluorescent staining of wild-type animals with antibodies against SYD-2 revealed distinct subcellular expression patterns in neurons and muscles (Fig. 3a–d). Using a *syd-2*-GFP reporter construct that expressed GFP in cell bodies (pCZ#14; Fig. 2a), we determined that *syd-2* was expressed in all neurons and muscles. Two lines of evidence indicate that *syd-2* is required in