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Testing Hamilton's rule with competition between relatives

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Hamilton's^{1,2} theory of kin selection suggests that individuals should show less aggression, and more altruism, towards closer kin. Recent theoretical work has, however, suggested that competition between relatives can counteract kin selection for altruism^{3–11}. Unfortunately, factors that tend to increase the average relatedness of interacting individuals—such as limited dispersal—also tend to increase the amount of competition between relatives. Therefore, in most natural systems, the conflicting influences of increased competition and increased relatedness are confounded, limiting attempts to test theory^{4,8–10}. Fig wasp taxa exhibit varying levels of aggression among non-dispersing males that show a range of average relatedness levels. Thus, across species, the effects of relatedness and competition between relatives can be separated. Here we report that—contrary to Hamilton's original prediction^{1,2,12} but in agreement with recent

theory^{5–11}—the level of fighting between males shows no correlation with the estimated relatedness of interacting males, but is negatively correlated with future mating opportunities.

Hamilton's rule^{1,2} provides a tool for understanding a range of social interactions, including altruism, aggression, selfishness and spite. It states that altruism (or less aggression) is favoured when $rb - c > 0$, where c is the fitness cost to the altruist, b is the fitness benefit to the beneficiary and r is their genetic relatedness. For a given benefit and cost, the evolution of altruism therefore relies upon a sufficiently high relatedness between interacting individuals. Hamilton² originally suggested that a high relatedness could arise in two ways: (1) behaviour based upon direct kin recognition between individuals, or (2) limited dispersal (population viscosity).

However, the importance of limited dispersal in increasing the relatedness among interacting individuals and favouring altruism has been controversial^{3–11}. Hamilton's original suggestion has been contested because limited dispersal can also increase competition between neighbouring relatives, which opposes the evolution of altruistic behaviour^{3–11}. Unfortunately, empirical tests of theory, that determine the relative importance of increases in both relatedness and competition between relatives, have been hindered because both factors are influenced by dispersal, and so their effects are usually confounded^{14,8–10}.

The variable form of mate competition and population structure across fig wasp species with wingless males offers an opportunity for disentangling the confounded effects of relatedness and competition between relatives in viscous populations^{12–18}. Fig wasps are species that develop within the fruit of fig trees, and include mutualistic pollinating species as well as parasitic non-pollinating species¹⁵. In many species the males are wingless, and mate with the winged females before the females disperse. The level of aggression between these non-dispersing males varies enormously across species^{12–15,18}. At one extreme, males of some non-pollinating species are highly modified for combat with armoured bodies and huge mandibles. These mandibles are used to tear soft tissue and sever body parts, including limbs, head and abdomen, and can result in extremely high mortality levels. At the other extreme, males of other non-pollinating and most pollinating species show no modifications for combat or aggression.

Across these species, the average relatedness of competing males varies enormously owing to variation in the number of females that lay eggs in each fruit^{12–16}. For example, if only one female lays eggs in a fruit then all the competing males will be brothers; increasing numbers of females laying eggs in a fruit will lead to males

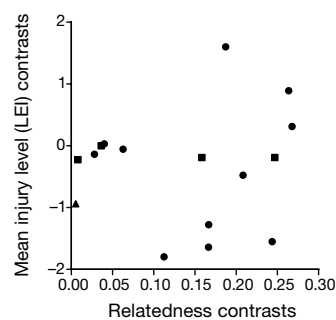


Figure 1 Mean injury level contrasts plotted against estimated relatedness contrasts. Across species, the mean injury level (lifetime extent of injury, LEI) and the proportion of individuals severely injured (SI) showed no significant relationship with estimated relatedness (LEI: all contrasts, $F(1,15) = 1.01$, $r^2 = 0.06$, $p = 0.33$; not including contrasts within the pollinator lineage, $F(1,11) = 0.72$, $r^2 = 0.06$, $P = 0.42$; SI: all contrasts, $F(1,15) = 0.04$, $r^2 < 0.01$, $p = 0.84$; not including contrasts within the pollinator lineage, $F(1,11) = 0.05$, $r^2 < 0.01$, $p = 0.83$). Circles, contrasts between the non-pollinating species; squares, contrasts between the pollinator species; triangle, the contrast between pollinators and non-pollinators.

competing with unrelated individuals^{12–16}. Hamilton¹² suggested that the variation in the level of fighting across fig wasp species (and other insects with fighting males) reflects different levels of relatedness: specifically, that kin selection favours altruism and less aggression in species in which competing males are more highly related. However, this prediction did not take into account the potentially conflicting effect of competition between relatives.

Wingless male fig wasps represent an extreme case with respect to competition between relatives because there is no dispersal before competition (competition is completely local)^{9–11}. More recent theory suggests that in this case competition between relatives totally removes the kin-selected benefits of altruism towards relatives^{5–11}, and so increased relatedness will not favour lower levels of aggression. Instead we predict that fighting levels should correlate with the importance of fighting over (and therefore mating with) any particular female^{13,17}, and so be negatively correlated to the total number of females developing in a fruit.

We estimated fighting levels and average relatedness between competing males in 25 fig wasp species (see Methods). Fighting levels were estimated by scoring wingless males for injuries after females had left the fruit¹³. Individual injuries were rated on a scale of 0–8 (for example, loss of an antenna scored 0.5 points, whereas loss of head with evisceration scored 8.0 points)¹³. The overall lifetime extent of injury (LEI) score for each individual was found by adding together the scores from its different injuries, subject to a maximum of 8.0. Individuals that had injuries rated at 8 points were classed as severely injured (SI). We used the sex ratio (proportion of males) to estimate average relatedness between competing males for the different wasp species (see Methods). Abundant evidence in pollinating and non-pollinating fig wasps suggests that the sex ratio of a species correlates with the number of females that lay eggs per fruit, and therefore the relatedness of individuals within fruits^{15,16}.

Previous comparative studies of fighting levels between males across fig wasp species^{12,14,18} have been hindered by the problem that male morphology, levels of fighting and the possible correlates of fighting levels (relatedness, male and female density) are tightly linked to phylogeny^{14–16,18}. Pollinating species (subfamily Agaoninae) generally develop in large broods, and competing males are often highly related and do not fight violently. In contrast, non-pollinating species (subfamilies Epichrysomallinae, Otitesellinae, Sycococinae, Sycoryctinae and Sycophaginae) frequently develop in smaller broods, competing males can be less related and, in several cases, exhibit violent fighting. This is a prime model for when

phylogenetic differences may lead to misleading correlations¹⁹; the potential danger of this is clearly shown by the fact that subfamily is able to explain greater than 50% of the variation in fighting levels across species. We address this potential problem by using a formal comparative method (independent contrasts¹⁹) based upon a molecular phylogeny that we have estimated (see Methods).

Controlling for phylogeny we found that both the mean LEI and the proportion of SI individuals showed no significant relationship with estimated relatedness (Fig. 1). In contrast, both LEI and the proportion of SI individuals were significantly negatively correlated with the mean number of females developing in a fruit (Fig. 2), as predicted by contest models which do not include any effect of relatedness^{13,17}. Thus, while the sex ratio of the wasps is correlated to relatedness and possible outcrossing^{10,12,15,16}, the form of mate competition between males responds to a different aspect of population structure—the number of females developing in a fruit. The robustness of our results is supported by the fact that the same results were also observed when the data were analysed without the contrasts derived within the pollinator lineage (where no fighting has been observed in the species we considered; Fig. 2). Furthermore, when testing for an effect of relatedness, our comparison across species provides more power than a comparison within a species (across figs), which would also require individuals to be able to assess their relatedness to other males in the fruit (some form of kin recognition) and adjust their behaviour accordingly.

Our results support the prediction that, with limited dispersal, the increased competition between relatives can negate the effect of increased relatedness in favouring altruism^{5–11}. One way¹⁰ of incorporating this into Hamilton's rule ($rb - c > 0$) is by expressing the marginal benefit of increased altruism, b , as a function of three parameters: $b = B - a(B - c)$ (Fig. 3; for alternative methods which focus on how relatedness is measured, see ref. 9). Here c has the standard meaning of the cost of altruism to the actor, and B is the benefit that would accrue to the recipients if the recipients did not compete with each other. The parameter a is the spatial scale at which competition occurs: an increase in the reproductive success of neighbours by a proportion x increases local competition by a factor ax , but has negligible effect on the intensity of global competition because the local neighbourhood is only a small part of the total population¹⁰. The parameter a therefore measures the extent to which neighbours (and potentially relatives) compete. If competition is completely global ($a = 0$) then any competition between relatives is negligible, and so the classic equation for Hamilton's rule

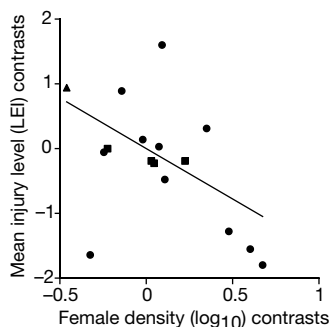


Figure 2 Mean injury level (LEI) contrasts plotted against the mean number of females developing in a fruit (\log_{10} transformed) contrasts. Across species, the mean injury level (LEI) and the proportion of individuals severely injured (SI) were significantly negatively correlated with the mean number of females developing in a fruit (\log_{10} transformed) for the analyses containing all contrasts; LEI: all contrasts, $F(1,15) = 5.92$, $r^2 = 0.28$, $P = 0.03$; not including contrasts within the pollinator lineage, $F(1,11) = 6.84$, $r^2 = 0.38$, $P = 0.02$; SI: all contrasts, $F(1,15) = 3.01$, $r^2 = 0.17$, $P = 0.10$; not including contrasts within the pollinator lineage, $F(1,11) = 5.00$, $r^2 = 0.31$, $P = 0.047$. See Fig. 1 for symbol (circles, squares and triangle) definitions.

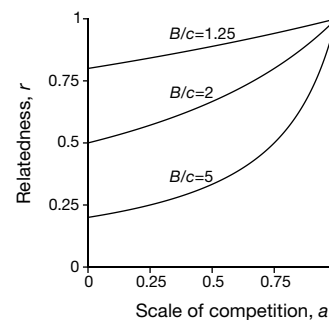


Figure 3 Hamilton's rule with relatedness and competition between relatives. Plotted is the minimum relatedness (r) required to favour altruism, against the scale of competition (a) (ref. 10). Competition varies from completely global ($a = 0$) to completely local ($a = 1$). The different lines represent different ratios of B/c , where B is the fitness benefits to the beneficiaries in the absence of competition between relatives, and c is the cost of altruism to the actor. We note that: (1) as competition becomes more local (greater competition between relatives; higher a), the relatedness (r) or relative fitness benefit to the beneficiary (B/c) must increase in order to favour altruism; (2) when competition is completely local ($a = 1$) altruism cannot spread ($r > 1$ is required).

holds^{9,10}. As competition becomes more local (increasing a), any increase in reproduction of a neighbour comes at a greater cost to other neighbours. This increases competition between relatives and so reduces the kin selection advantage in being altruistic (reduces b). In the extreme, with competition being completely local ($a = 1$), as is the case with competition for mates between wingless male fig wasps, the increased competition between relatives exactly cancels the advantage of increased relatedness. At this point any increase in the reproduction of a neighbour must come at the cost of other related neighbours, and so altruism is not favoured^{9,10}. Further support for this prediction comes from the observation that, in other insect cases in which closely related males show extreme aggression, competition for mates tends to be very local ($a \rightarrow 1$)¹².

More generally, our results emphasize the importance of determining the amount of competition between relatives, and that estimating the scale at which competition occurs (a) can be as crucial as estimating relatedness (r). Whereas the importance of relatedness is well appreciated, and frequently estimated, the scale at which competition occurs (a) is generally ignored, let alone quantitatively estimated. Although this may partly reflect difficulties in empirical estimation of a , its effect may be incorporated into Hamilton's rule in several ways^{9,10}. When competition between relatives is ignored, the importance of kin selection is overestimated. Furthermore, there are a number of recent studies that fail to show a correlation between relatedness and altruism²⁰, including behaviours such as cannibalism (insects²¹), cooperative breeding (meerkats and several bird species^{20,22,23}) and watching for predators (meerkats²⁴). We suggest that some of these cases might be explained by the fact that there is enough competition between relatives to ensure that kin selection benefits are relatively unimportant (sufficiently high a). Consistent with this possibility is that, in all of these cases, an individual (direct) fitness explanation can be provided for the behaviour in question and its variation. □

Methods

Data collection

Data on 25 species of fig wasps had been collected from seven species of Malaysian wild fig^{13,14}. The wasp species consist of eight pollinator species, and 17 non-pollinator species. Almost-ripe fruit were collected, from which wasps were about to emerge. The fruit were enclosed in jars with cloth lids, and after the wasps emerged, the contents of each jar were preserved in 75% alcohol. Later, all the wasps which had and had not emerged from the fruit were identified to species, sexed, counted and the males scored for the injuries that they obtained in their lifetime (termed lifetime extent of injury, LEI). Individual injuries were rated on a scale of 0–8 (loss of part or whole antenna, 0.5 points; loss of part or whole tarsus or small bruise, 1.0; loss of part or whole tibia (plus tarsus) or bruise with cut, 2.0; loss of part or whole femur (plus tibia and tarsus) or large bruise or bruise plus crushed area, 3.0; loss of part or whole coxa (plus femur, tibia and tarsus) or half severed abdomen or head with no evisceration, 4.0; >half severed abdomen or head with evisceration, 8.0)¹³.

We used the sex ratio (proportion of males) to estimate average relatedness between competing males for the different wasp species. In both pollinating and non-pollinating fig wasps, the sex ratio varies with the number of females that laid eggs in a fruit, and therefore average relatedness, as predicted by sex ratio theory^{12,15,16}. The fewer females that lay eggs in a fruit, the more female biased their offspring sex ratio is. Quantitative estimates of mean relatedness between wasps within a fruit can be obtained by rearranging a well-known expression for the expected (unbeatable) sex ratio (the proportion of males, m) as a function of relatedness (r in haplodiploid species^{10,12,15,16}), to give r as a function of m : $r = 4(1 - m)/(3 - m + \sqrt{1 + 10m + m^2})$. More generally, the use of sex ratio data to estimate relatedness has gained quantitative support from work on other organisms (malaria and related protozoan parasites), where estimates of relatedness derived from sex ratios have been confirmed by estimates derived directly from genotypes^{25,26}.

Phylogeny

A molecular phylogeny was estimated using nucleotide sequences 816 base pairs long collected from the 3' end of the cytochrome oxidase subunit I gene (COI)^{27,28}. Analyses were performed with version 4.0b1 of PAUP*, written by D. L. Swofford (Smithsonian Institution, Washington, DC). Our molecular data set contained 15 species of fig wasps that included either the species scored for male injury level, or species from the same genera or subfamily (GenBank accession numbers: AF302052–AF302067). Sequences from the following species were used in the phylogenetic analyses: *Ceratosolen constrictus*, *Ceratosolen solmsi* (same species scored for male injury level); *Blastophaga nipponica*, *Eupristina verticillata*, *Ceratosolen galili*, *Eujacobsonia* sp. (light trap, unknown host), *Apocrypta* sp. (ex. *Ficus sycomorus*), *Sycoricetes* sp. (ex. *Ficus glumosa*), *Sycoscapter* sp.

(ex. *F. cerecarpa*), *Philotrypesis* sp. (ex. *Ficus tinctoria*), *Eukoebalea* sp. (ex. *F. sycomorus*) (species of the same genera as those scored for injury level); *Heterandrium* sp. (ex. *Ficus dugandii*) (non-pollinator from subfamily Otitesellinae). Additionally, sequences from one species of the African genus *Apocryptophagous* (ex. *F. sycomorus*) and two species from the neotropical genus *Idarnes* (ex. *Ficus trigonata*, *Ficus obtusifolia*) were used in the phylogenetic analyses to balance the uneven sampling from different subfamilies. Maximum likelihood methods were used to reconstruct the phylogeny. The general reversible model with rate heterogeneity was used²⁹, and the parameters of the model were estimated from the data. The tree topology was estimated using an heuristic algorithm with branch swapping (tree bisection–reconnection). The heuristic search was repeated three times and the consensus tree from the best three trees was used for generating the final phylogeny (see Supplementary Information). The species scored for male injury level that were not present in the molecular phylogeny were added to the nodes leading to species of the same genus or subfamily.

Statistical analyses

The data were analysed using phylogenetically independent contrasts. These were derived by calculating the difference in the response and explanatory variables across pairs of species or higher nodes that share a common ancestor¹⁹, as implemented in the CAIC statistical package³⁰. The continuous explanatory variables used were those that theory suggests may be important: the average number of males in a fruit, the average number of females in a fruit, the ratio of average number of females to average number of males, 1/(the average number of females times the number of males), and estimated relatedness. The explanatory powers of these different variables were tested without transformation, log₁₀ transformed, and inverted. Our phylogeny led to 16 contrasts from the 25 species. These contrasts were then analysed with multiple regressions through the origin¹⁹. In all analyses we initially fitted a full model, including all explanatory variables that we were considering. Terms were then removed from the model by stepwise deletion. Across species, the absolute (untransformed) ranges for the various variables were: number of females developing in a fruit (1.7–574.5); LEI (0.046–5.496); SI (<0.01–0.48). The mean number of females developing in a fruit showed no significant relationship with the mean sex ratio ($F(1,23) = 1.31, r^2 = 0.05, p = 0.26$).

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Reduced antinociception and plasma extravasation in mice lacking a neuropeptide Y receptor

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Neuropeptide Y (NPY) is believed to exert antinociceptive actions by inhibiting the release of substance P and other 'pain neurotransmitters' in the spinal cord dorsal horn^{1–3}. However, the physiological significance and potential therapeutic value of NPY remain obscure⁴. It is also unclear which receptor subtype(s)

are involved. To identify a possible physiological role for the NPY Y1 receptor in pain transmission, we generated NPY Y1 receptor null mutant (Y1^{-/-}) mice by homologous recombination techniques. Here we show that Y1^{-/-} mice develop hyperalgesia to acute thermal, cutaneous and visceral chemical pain, and exhibit mechanical hypersensitivity. Neuropathic pain is increased, and the mice show a complete absence of the pharmacological analgesic effects of NPY. In the periphery, Y1 receptor activation is sufficient and required for substance P release and the subsequent development of neurogenic inflammation and plasma leakage. We conclude that the Y1 receptor is required for central physiological and pharmacological NPY-induced analgesia and that its activation is both sufficient and required for the release of substance P and initiation of neurogenic inflammation.

Homologous recombination in embryonic stem cells was used to establish mice deficient in the NPY Y1 receptor. The disruption was generated by introducing an internal ribosomal entry site followed by a Tau–LacZ fusion minigene into the second exon of Y1 (Fig. 1a). Southern blot analysis confirmed that the Y1 allele was disrupted and northern blot analysis showed that instead of the messenger RNA transcripts encoding Y1, the mutant (Y1^{-/-}) mice produced the expected mRNA encoding β-galactosidase (Fig. 1b–d). As previously described, female Y1^{-/-} mice display a late-onset overweight compared to their littermates⁵ (data not shown). Y1 receptors are abundant in the forebrain, whereas little or nothing is present in the brainstem⁶. Y1 receptors are also highly expressed in dorsal root ganglion neurons in preferentially small and medium size neurons^{6,7}. However, the central termination of Y1 nerve fibres in the dorsal horn, and whether Y1 is expressed in both of the two major cytochemical subpopulations of pain neurons, the SP peptidergic and non-peptidergic pain neurons⁸, is unresolved.

β-galactosidase histochemical and immunohistochemical staining of spinal cord sections from Y1^{-/-} mice showed strong staining localized exclusively to the dorsal horn (Fig. 1e, f). Immunohistochemical double staining for β-galactosidase (staining Y1 expressing neurons and fibres) and the lectin IB4 (staining somas and nerve fibres of unmyelinated non-peptidergic sensory nociception neurons⁸) showed a strong staining for Y1 nerve fibres in dorsal

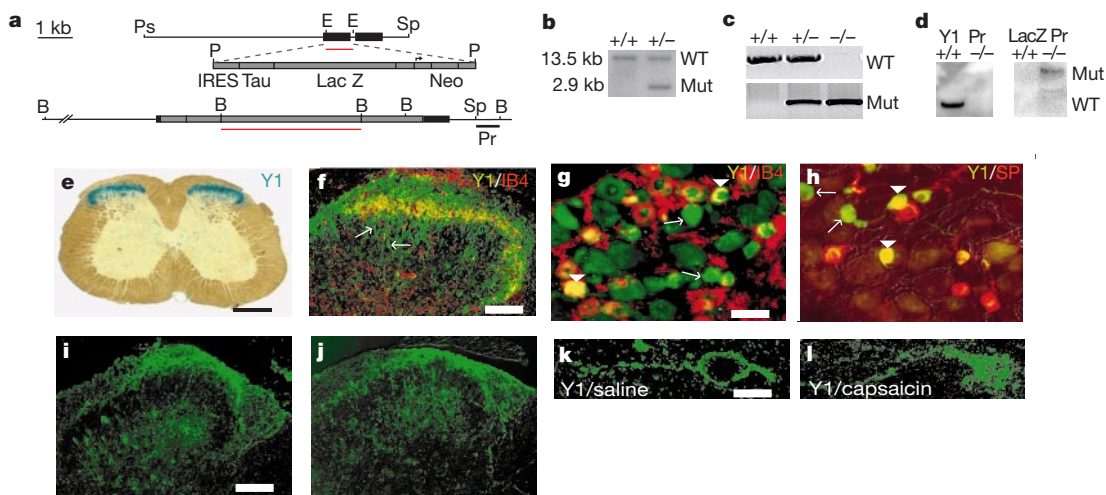


Figure 1 Targeted mutagenesis of the Y1 receptor and expression analysis of Y1 and SP receptors. **a**, Y1 gene targeting. Top, targeting vector (black boxes, Y1 coding exons). The disrupting cassette is indicated. Bottom, restriction map of the resulting targeted allele (B, BamHI; Sp, SpeI; E, EcoRI; P, PacI; Pr, probe used in the Southern blots.). **b**, Southern blot of ES cells. **c**, PCR genotyping of wild-type, Y1^{+/-} and Y1^{-/-} mice. **d**, Northern blot of total brain RNA of Y1^{+/+} and Y1^{-/-} mice using a Y1 probe (Y1 Pr) or LacZ probe (LacZ Pr). These probes are underlined in red in **a**. **e**, Transverse section from the spinal cord lumbar enlargement of Y1^{-/-} mice histochemically stained for β-galactosidase. **f**, Y1^{-/-} mice immunohistochemically stained for β-galactosidase-positive nerve terminals and

neurons (arrows) in the spinal cord dorsal horn (green) and the lectin IB4 (red, layer I liner). **g**, **h**, Double staining of L4 dorsal root ganglion for β-galactosidase (green) and IB4 (red) (**g**), and for β-galactosidase (green) and SP (red) (**h**). Arrows point to single-stained neurons, arrowheads to double-stained neurons. **i**, **j**, SP receptor distribution in the dorsal horn of wild-type mice (**i**) and Y1^{-/-} mice (**j**). **k**, SP receptor staining in lamina I of the contralateral vehicle injected side of Y1^{-/-} mice. **l**, Loss of cell surface and increase of intracellular SP receptor immunoreactivity in lamina I 10 min after capsaicin injection into the hindpaw of Y1^{-/-} mice. Scale bar, 300 μm (**e**), 80 μm (**f**, **i**, **j**), 30 μm (**g**, **h**), 20 μm (**k**, **l**).