

Self-recognition, color signals, and cycles of greenbeard mutualism and altruism

Barry Sinervo*^{†‡}, Alexis Chaine*, Jean Clobert^{†§}, Ryan Calsbeek*[¶], Lisa Hazard*^{||}, Lesley Lancaster*, Andrew G. McAdam*^{***}, Suzanne Alonzo*^{††}, Gwynne Corrigan*, and Michael E. Hochberg*^{††§§}

*Department of Ecology and Evolutionary Biology, University of California, Earth and Marine Sciences Building, Santa Cruz, CA 95064; [†]Laboratoire D'Ecologie, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 7625, Bâtiment A, Emé Etage, Case 237, 7 Quai Saint Bernard, F-75252 Paris Cedex 05, France; [§]Laboratoire Evolution et Diversité Biologique, Station Biologique du Centre National de la Recherche Scientifique Moulis, 09200 Saint-Girons, France; [¶]Center for Tropical Research, Institute of the Environment, University of California, 1609 Hershey Hall, Los Angeles, CA 90095; ^{||}Department of Biology and Molecular Biology, Montclair State University, 1 Normal Avenue, Upper Montclair, NJ 07043; ^{***}Department of Fisheries and Wildlife and Department of Zoology, Michigan State University, 13 Natural Resources Building, East Lansing, MI 48824; ^{††}Department of Ecology and Evolutionary Biology, Yale University, 427 Osborn Memorial Laboratory, New Haven, CT 06520-8106; ^{†††}Institut des Sciences de l'Evolution, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 5554, Université Montpellier II, 34095 Montpellier, France; and ^{§§}Santa Fe Institute, 1399 Hyde Park Road, Santa Fe, NM 87501

Edited by David B. Wake, University of California, Berkeley, CA, and approved March 7, 2006 (received for review November 28, 2005)

Altruism presents a challenge to evolutionary theory because selection should favor selfish over caring strategies. Greenbeard altruism resolves this paradox by allowing cooperators to identify individuals carrying similar alleles producing a form of genic selection. In side-blotched lizards, genetically similar but unrelated blue male morphs settle on adjacent territories and cooperate. Here we show that payoffs of cooperation depend on asymmetric costs of orange neighbors. One blue male experiences low fitness and buffers his unrelated partner from aggressive orange males despite the potential benefits of defection. We show that recognition behavior is highly heritable in nature, and we map genetic factors underlying color and self-recognition behavior of genetic similarity in both sexes. Recognition and cooperation arise from genome-wide factors based on our mapping study of the location of genes responsible for self-recognition behavior, recognition of blue color, and the color locus. Our results provide an example of greenbeard interactions in a vertebrate that are typified by cycles of greenbeard mutualism interspersed with phases of transient true altruism. Such cycles provide a mechanism encouraging the origin and stability of true altruism.

alternative strategies | linkage map | frequency-dependent selection | evolutionarily stable strategy | cooperation

The evolutionary stability of cooperative and altruistic behaviors requires that interindividual benefits be protected from competition, cheating, and defection (1–4). Without such safeguards, selfish strategies will eliminate altruistic strategies (5, 6). Hamilton (5) theorized that true altruism might evolve if a supergene simultaneously affected a signal and recognition of the signal and that signal recognition elicited social acts costly to donors but beneficial to recipients. Dawkins (6) coined Hamilton's social supergene a greenbeard in a hypothetical example of altruists that sported a green beard distinct in color from other beards sported by nonaltruists. Despite studies consistent with greenbeard altruism (7–12), few provide definitive evidence for greenbeard altruism.

The annual side-blotched lizard, *Uta stansburiana*, exhibits six color genotypes (13, 14) (*oo*, *bo*, *yo*, *bb*, *by*, and *yy*), which serve as markers for three male strategies (15). Orange males (*oo*, *bo*, and *yo*) usurp territory. Blue males (*bb*) mate-guard. Yellow males (*by* and *yy*) are sneakers. Male competition drives rock–paper–scissors (RPS) cycles of three strategies: sneakers beat usurpers, mate guards defeat sneakers, and usurpers prevail over mate guards (13, 15–19). Previously, we showed that males with *b* alleles prefer to settle near non-kin but genetically similar *bb* males and cooperate in territory defense (15). Hereafter, we refer to *bb* males with genetically similar neighbors (based on allele sharing at nine microsatellite loci) as “dyadic *bb* pairs” (15). We contrast dyadic *bb* males with “loner *bb* males” that may have *bb* neighbors, but none are genetically similar. The fitness of loner *bb* with ($n = 53$) and without ($n = 9$) *bb* neighbors does not differ ($F_{1,57} = 0.11$; $P > 0.7$, factor for year, not significant), so we pooled them as loner *bb*

males. Here we test whether dyadic pairs satisfy criteria of altruism in which one male bears a fitness cost that benefits an unrelated male ($W_{\text{beneficiary}} > W_{\text{loner}} > W_{\text{altruist}}$). We distinguish such greenbeard altruism from mutualism ($W_{\text{beneficiary}} > W_{\text{altruist}} > W_{\text{loner}}$) in which both dyadic males have higher fitness than loners.

To determine whether the *b* allele contributes to greenbeard altruism or mutualism, we measured fitness payoffs of male strategies (siring success and progeny recruits) with DNA paternity (18). Because few males survive more than 1 year, this measures lifetime reproductive success.

We also used the field pedigree for *Uta* to construct a linkage map of heritable behaviors for self-recognition of genetic similarity and the OBY color locus. We identify specific genes, distributed across the genome, that are responsible for self-recognition and settlement behavior of *bb* males and females.

Results and Discussion

Dyad Formation and Kin Philopatry. A key component of non-kin greenbeards is the ability of unrelated individuals to find a partner. We used microsatellite loci to determine which neighbors share enough alleles (e.g., genetically similar) such that they could be sire–progeny pairs or sibs (13–18). A sire–progeny criterion of relatedness ($r = 0.5$) is more stringent than that of natural half sibs ($r = 0.25$) given the polyandrous mating system (18). Precise maternal pedigrees provided definitive evidence that cooperating males are unrelated. Although a greenbeard is not incompatible with kin selection, its action potentially confounds genic selection with kin nepotism. We tested for kin philopatry in associations of genetically similar males.

Genetically similar *bb* neighbors occur at ≈ 3.4 times the expected rate (15 pairs observed, 4.4 expected; $\chi^2 = 20.8$, 1 d.f., $P < 0.0001$) on sib-randomized plots. Dyadic males are unrelated based on the maternal pedigree (15). Blue males do not preferentially settle close to other B males, relative to O and Y males ($F_{2,256} = 3.14$, $P < 0.05$; number of B neighbors: $B = 1.58 \pm 0.15$, $Y = 1.47 \pm 0.14$, $O = 2.10 \pm 0.22$; $B = Y < *O$, $P < 0.05$, in post hoc tests). Thus, self-recognition and settlement of genetically similar *bb* males are determined by more than just blue color. Experimental randomization of sibs in this sample may have precluded kin groups from forming, biasing settlement in favor of unrelated *bb* male dyads. Thus, if males use phenotype matching to find and cooperate with kin, observed settlement of unrelated yet genetically similar males may be an artifact of kin randomization.

Conflict of interest statement: No conflicts declared.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: RPS, rock–paper–scissors; LD, linkage disequilibrium.

[†]To whom correspondence should be addressed. E-mail: sinervo@biology.ucsc.edu.

© 2006 by The National Academy of Sciences of the USA

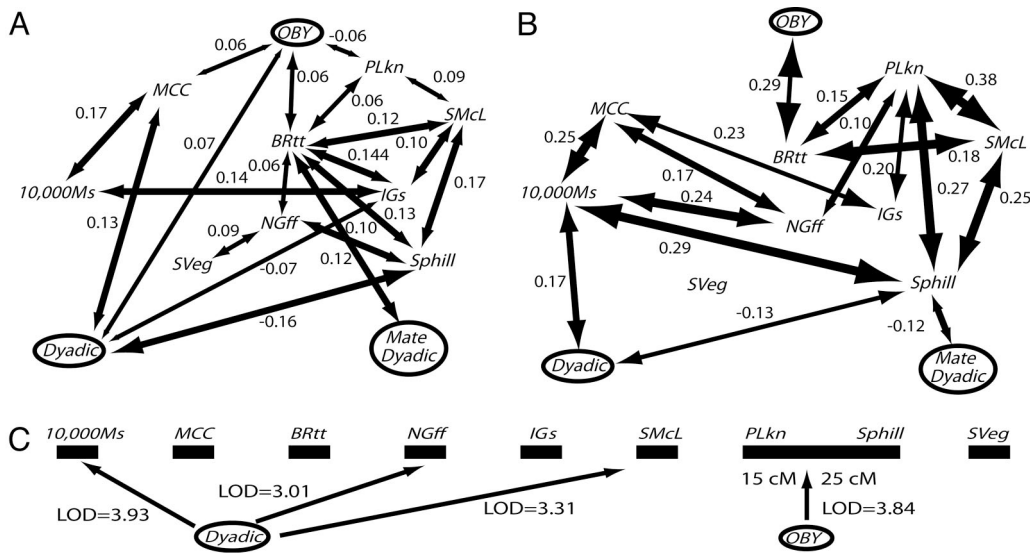


Fig. 1. Genomic effects of correlational selection on male throat color strategies and a linkage map of the color signal (OBY) and self- and color-recognition behavior. Effect of correlational selection during altruistic (A) and mutualistic (B) phases on genome-wide LD (r values by arrows). Genome-wide LD is ≈ 3 -fold greater during mutualistic phases [1998: index of association (IA) = 0.94, average disequilibrium (rD) = 0.087, $P < 0.01$, $n = 52$ males] than during altruistic phases (1992: IA = 0.28, average rD = 0.026, $P < 0.01$, $n = 127$ males). The OBY locus and self-recognition behavior (of both sexes) for genetically similar *bb* males are both in LD with microsatellite loci. (C) Primary linkage map of microsatellite loci based on markers in the field pedigree that are on separate blocks mapped at 100 Kosami cM. Genetic factors for OBY and three factors for dyadic self-recognition behavior (sexes pooled) are on separate linkage groups.

A large-scale experiment in which progeny were released on sibship-intact plots tested specifically for kin philopatry in forming associations of genetically similar males. On sibship-intact plots, five genetically similar *bb-bb* dyads formed, two *bb-by* dyads formed, and one *bo-yo* dyad formed. No dyads were kin based on the pedigree. Thus, 10 *bb* males (five pairs) formed dyads of 35 *bb* males reflecting 29% of all *bb* males, comparable to that observed on sib-randomized plots (33%). This finding reflects a nonrandom pattern largely restricted to *bb* males (dyadic *bb*: 5 pairs observed, 0.5 expected; dyadic non-*bb*: 3 pairs observed, 6.9 expected; $\chi^2 = 43.3$, 1 d.f., $P < 0.0001$). Thus, presence of kin has no effect on formation of *bb* dyads among genetically similar males at maturity.

To determine why kin philopatry does not contribute to *bb* dyad formation, we compared survival and dispersal on sibship-intact and randomized plots. On sibship-randomized plots we recovered 58 males at maturity of 424 neonates including seven sib pairs. On sibship-intact plots we recovered 58 males at maturity of 910 neonates and only three sib pairs (only 1 male was *bb*). On sibship-intact plots, none of the three sib pairs formed dyads because they settled at an average distance [25.5 ± 6.5 m (SE)] greater than between dyadic *bb* cooperators (< 10 m). Survival of sib pairs was higher on sib-randomized than sib-intact plots (14/424 vs. 6/910; $\chi^2 = 13.7$, 1 d.f., $P < 0.001$). Higher mortality on sibship-intact plots suggests that hatchling kin competition favors dispersal, not philopatry, posing a constraint on kin cooperation. Dyads are thus more likely to form between unrelated but genetically similar males.

Associations of *bb* dyads could arise from mutual attraction to environmental features (e.g., rocks or *bb* females) rather than from greenbeard recognition. Four lines of evidence indicate that this is not the case. First, dyads lack consistency in location among years ($n = 15$, 8, and 7 dyads in 1992, 1998, and 1999). Minimum distance between nearest dyads across years averaged 18.8 ± 2.7 m. Second, manipulating a likely point of attraction, large rock piles (20, 21), does not affect *bb* dyad formation. Half of the *bb* dyads formed > 10 m away from piles ($n = 7$ of 14), and 21 of 38 *bb* males settled > 10 m from piles. Third, dams with *b* alleles were less likely to overlap territories with *bb* dyads than loners [1.16 ± 0.38 vs. 1.98 ± 0.25 (SE); $F_{1,25} = 3.34$, $P = 0.07$]. Dyads are not attracted to overall

female density; dyadic *bb* males had fewer females per territory than loner *bb* males (1.77 ± 0.38 vs. 2.64 ± 0.21 females; $F_{1,117} = 3.93$, $P = 0.05$). Fourth, *bb* dyads are genetically similar across nine microsatellite loci, not just OBY, implying a genetic cause.

Genomic Architecture of Self-Recognition Behavior and the OBY Color Locus. Cosettlement of dyadic *bb* males implies that *b* color-recognition and self-recognition (e.g., genetic similarity) should have a simple genetic basis. We found that *b* dyadic recognition was heritable (1998–1999, $h^2 = 0.97 \pm 0.30$; mid-parent regression: $F_{1,29} = 12.26$, $P < 0.002$) and similar for sons ($h^2 = 0.89$) and daughters ($h^2 = 1.05$) (analysis of covariance: $F_{1,29} = 0.70$, $P = 0.51$). Thus, females and males share self-recognition alleles, which can promote assortative mating in females for dyadic *bb* males (B.S., unpublished data). High heritability implies alternative alleles besides self-attraction, which could include “self-repulsion” or “neutral” settlement behaviors with respect to genetically similar neighbors. Such alleles could explain previously documented patterns of O hyperdispersion and Y settlement (15).

High heritability also implies that genetic factors underlying color and self-recognition can be mapped to microsatellite markers with decay of linkage disequilibrium (LD) in progeny, given high LD among microsatellite loci and dyadic behavior (Fig. 1A and B). We constructed a primary linkage map and then mapped (Fig. 1C) genetic variation for OBY color alleles and self-recognition of *bb* dyads. The three alleles of the OBY locus (14) mapped to a single genetic factor between *Plkn* and *Sphil*. In contrast, self-recognition behavior of genetically similar males with *b* alleles mapped to three linkage groups (10000M, *NGff*, and *SmcL*), implying that high heritability for self-recognition and cosettlement of dyadic *bb* males and nonrandom mating of dams with dyadic *bb* sires is due to at least three unlinked genetic factors. Our results therefore indicate that the “greenbeard” corresponds to self-recognition of multiple loci including, but not limited to, *b* alleles of the OBY locus: highly successful *bb* cooperators must share alleles at all four loci.

Fitness Costs and Benefits of *bb* Dyads and the RPS Cycle. For altruism to occur, participating individuals must find a suitable partner, and

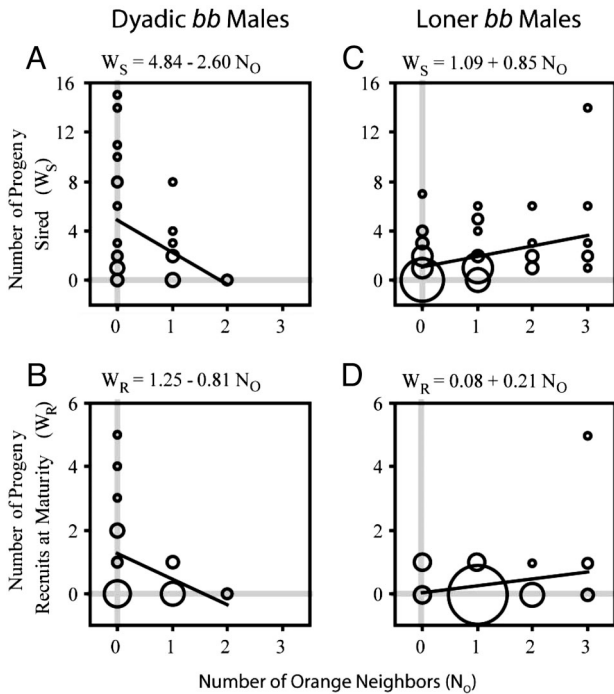


Fig. 2. Fitness effects of crowding by O males on dyadic and loner *bb* males. Siring success is significantly related to number of O neighbors (N_O) for dyadic and loner *bb* males ($P < 0.05$ all regressions; analysis of covariance: differences in slope significant; $W_S: F_{1,93} = 13.38, P = 0.0004$; $W_R: F_{1,93} = 12.60, P = 0.0006$). Data give parameter estimates for dyads (A, β_1 and β_2 ; B, β_1 and β_2) and loners (C, β_3 and β_4 ; D, β_3 and β_4) used in evolutionarily stable strategy (ESS) analysis.

a cost must result from their otherwise mutualistic interactions. Costs of altruism can be measured relative to a loner strategy that does not participate in this group behavior. Settlement of O males beside *bb* males reduces average fitness of dyadic *bb* relative to loner *bb* males (Fig. 2). However, reduced fitness of dyadic males is primarily borne by one male (Fig. 3). The male with many O neighbors has low fitness compared with the male with few O neighbors ($\Delta W_{\Delta N_O}$; Fig. 3). Even though loner *bb* males gain fitness from O neighbors, their strategy still loses to O genotypes that have higher siring success overall ($W_{S,loner\ bb} = 1.80 \pm 0.27, W_{S,bo} = 4.83 \pm 1.91, W_{S,oo} = 1.90 \pm 0.76, W_{S,yo} = 3.53 \pm 0.98$; analysis of covariance: $F_{1,114} = 3.43$; post hoc test: loner *bb* and *oo* significantly lower than *bo* and *yo*). This finding reflects the fact that O beats B in the RPS game (17–19).

In the notation of fitness costs and benefits, dyadic *bb* males with more O neighbors are donors and those with fewer O neighbors are

beneficiaries or recipients of social acts. Fitness asymmetries in dyads may be larger when the number of O males ($N_O[t]$) cycles to high frequency because of RPS dynamics (16–19), which leads to the interesting possibility that among-year changes of O frequency may alter social interactions among generations of *bb* males from altruism ($W_{recipient} > W_{loner} > W_{donor}$) to mutualism ($W_{recipient} > W_{donor} > W_{loner}$).

To determine whether *bb* dyads fit criteria for altruism or mutualism, frequency-dependent payoffs for recipients and donors were compared with loners using fitness relations (β_i parameters supplied in Figs. 2 and 3). Fitness of a strategy at time t ($W_j[t]$ where j = recipient, donor, or loner) is a function of $N_O[t]$ and fitness asymmetry, $\Delta W_{\Delta N_O}[t]$ (slope, Fig. 3), of dyads:

$$W_{recipient}[t] = \beta_1 + \beta_2 \times N_O[t] + \Delta W_{\Delta N_O}[t]/2, \quad [1]$$

$$W_{donor}[t] = \beta_1 + \beta_2 \times N_O[t] - \Delta W_{\Delta N_O}[t]/2, \text{ and} \quad [2]$$

$$W_{loner}[t] = \beta_3 + \beta_4 \times N_O[t]. \quad [3]$$

Costs and benefits of cooperation for dyads, $\Delta W_{\Delta N_O}[t]$, depend on $\Delta N_O[t]$, which arises from random asymmetries in O settlement next to dyadic males (Fig. 3A and B):

$$\Delta W_{\Delta N_O}[t] = \beta_5 \times \Delta N_O[t]. \quad [4]$$

$\Delta W_{\Delta N_O}[t]$ can be expressed in terms of $N_O[t]$ by noting that $\Delta N_O[t]$ depends on $N_O[t]$ (Fig. 3C), a relation that arises from small neighborhood effects on variance in number of O neighbors (19):

$$\Delta W_{\Delta N_O}[t] = \beta_5 \times (\beta_6 \times N_O[t]). \quad [5]$$

Eqs. 1–5, parameterized by field estimates of fitness (Figs. 2 and 3), allow us to calculate fitness of strategies over time (Fig. 4B), given $N_O[t]$ for *bb* dyads over 14 generations ($t = 1990$ –2003; Fig. 4A). In the long run, geometric mean (*GM*) (22) fitness of donor and loner males are similar, even though short-term fitness of donor males dips close to zero (Fig. 4; GM_{sired} : $W_{recipient} = 3.53, W_{donor} = 2.11, W_{loner} = 1.76$; $GM_{recruits}$: $W_{recipient} = 0.80, W_{donor} = 0.22, W_{loner} = 0.19$). If genetically similar *bb* males are available it pays to cooperate: *GM* fitness of dyads is higher than loner males ($GM_{sired}\{[W_{donor} + W_{recipient}]/2\} = 2.86, GM_{recruits} = 0.64$). Thus, the dyadic strategy is mutualistic in the long run (15), but high average fitness of greenbeard *b* alleles balances transient altruistic costs paid by donors when O is common. Both males should accept these risks of cooperation even though one will receive approximately zero payoff.

In ensuing phases of the RPS, O frequency falls because when common O is susceptible to the Y male strategy, which mimics females and cuckolds O males (16–18). In these years, *bb* males gain fitness from Y neighbors, but only if they form cooperative dyads of genetically similar *bb* males. Although frequency of Y neighbors

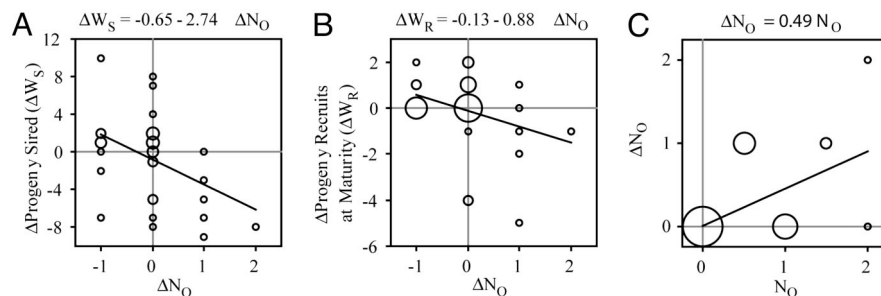


Fig. 3. Fitness effects of asymmetries in O male crowding on dyadic males and relationship of the asymmetry in O male crowding to average number of O males next to dyadic *bb* males. Difference in number of O male neighbors (ΔN_O) is related to difference in siring success (A) ($\Delta W_S: F_{1,26} = 4.82, P = 0.03$) and recruits (B) ($\Delta W_R: F_{1,26} = 4.82, P = 0.03$) of dyadic *bb* males. (C) ΔN_O depends on average number of O neighbors (N_O) for dyadic *bb* males ($F_{1,31} = 7.15, P = 0.01$). A and B give estimates for β_5 for W_S and W_R , respectively, and C is used to convert $N_O[t]$ to $\Delta N_O[t]$ (β_6).

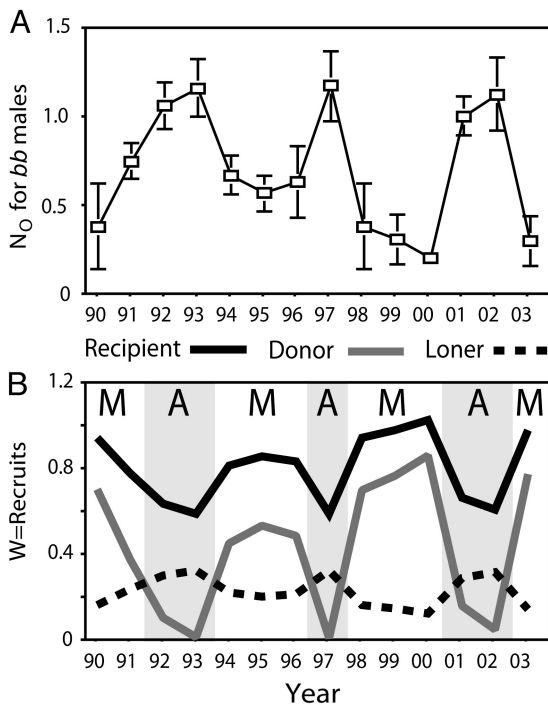


Fig. 4. RPS cycles drive cycles of mutualism and altruism in recipient and donor males of a dyadic pair relative to loner males. (A) Mean number of O neighbors (\pm SE) for *bb* males (N_O) as a function of RPS cycles (11, 12). (B) Progeny recruits for recipients, donors, and loners computed using Eqs. 1–5. RPS cycles drive $N_O[t]$, generating alternating phases of altruism (A) and mutualism (M).

(f_Y) does not affect fitness of loner *bb* males who only have genetically dissimilar neighbors (siring: $W_S = 2.22 - 0.97 \times f_Y$, $F_{1,64} = 0.73$, $P = 0.39$; recruits: $W_R = 0.42 - 0.35 \times f_Y$, $F_{1,64} = 0.73$, $P = 0.39$), f_Y enhances fitness of *bb* dyads ($W_S = 0.77 + 3.53 \times f_Y$, $F_{1,22} = 3.76$, $P = 0.06$, $W_R = 0.00 + 0.85 \times f_Y$, $F_{1,22} = 5.05$, $P = 0.03$). A significant difference between effects of f_Y on loner vs. dyadic *bb* males (W_S : $F_{1,82} = 6.06$, $P = 0.01$; W_R : $F_{1,82} = 4.32$, $P = 0.04$) indicates that f_Y affects the strategies differently. During the Y phase of the RPS, the *bb* greenbeard is mutualistic. As *bb* males invade during this mutualistic phase, conditions are reestablished for invasion of O males, thereby fueling a return to *bb* altruism. evolutionarily stable strategy (ESS) analyses (Eqs. 1–5) are sup-

ported by comparisons of average fitness of *bb* recipients, donors, and loners during mutualistic versus altruistic phases of the RPS cycle (Table 1). Therefore, dyad formation among genetically similar males is favored on average by mutualistic benefits to each *bb*. However, not all years are mutualistic: at high N_O , donor males have lower fitness relative to recipients and loner males.

Stability of Altruism in the Face of Egoists. Mutualism is stable from the perspective of an egoist; because both partners benefit there is little risk of defection. Altruism is not stable for donors who get low fitness. Donors should defect and opt for a higher payoff strategy if one exists. The low fitness of donor males when O is common indicates that donor males should use the presence of O neighbors as a cue to defect. However, defection requires that donor males have a viable recourse.

A viable defection strategy would be for donors to displace recipients, provided recipients become donors. If so, fitness asymmetry of dyadic males should be correlated with an asymmetry in resource holding potential. However, we found no effect of resource holding potential traits (21) on fitness asymmetry of donors and recipients (mass: $F_{1,26} = 1.02$, $P > 0.32$; condition: $F_{1,26} = 1.35$, $P > 0.26$).

Another viable recourse for donors includes partner abandonment for a higher-payoff territory (i.e., adopt loner strategy) when O males settle beside donors at the beginning of the breeding season (21–23). Such a relocation strategy is possible. Loner *bb* males can obtain a territory 2–3 weeks after most males (20, 22) and do not lose fitness relative to loners that remain stationary [$n = 8$ loners that moved ($W_S = 2.33 \pm 0.91$, $W_R = 0.35 \pm 0.32$) vs. $n = 54$ that were stationary ($W_S = 2.09 \pm 0.40$, $W_R = 0.20 \pm 0.14$); W_S : $F_{1,57} = 0.22$, $P > 0.64$, not significant; no year effect: $F_{1,57} = 0.43$, $P > 0.72$; W_R : $F_{1,57} = 0.06$, $P > 0.80$, not significant; no year effect: $F_{1,57} = 1.56$, $P > 0.20$). Potential payoffs of defection would be more appropriately measured for *bb* males that abandon dyads; however, no *bb* males that have formed a dyad with a genetically similar male have ever been observed to move (0/40) despite obvious fitness benefits that donors could obtain via defection. By contrast, loners can avoid fitness costs of inappropriate social neighborhoods by abandoning territories at a significantly higher rate (8/82; likelihood ratio = 6.63, $P < 0.01$, 1 d.f.) and finding new sites. Therefore, the donor is truly altruistic by foregoing potential benefits of defection in years with high N_O .

Evolution of Greenbeard Dynamics: A Synthesis. Donor males of *bb* dyads of side-blotched lizards are true altruists when O males are common in that they forgo potential benefits of defection.

Table 1. Observed payoffs for relative fitness

Years	$W_{\text{recipient } bb}$		$W_{\text{loner } bb}$		$W_{\text{donor } bb}$
Siring success ($W_{S,j}$)					
1992	1.93 \pm 0.62	>*	0.95 \pm 0.19	>*	0.41 \pm 0.26
1998–1999	2.71 \pm 0.65	>>*	1.57 \pm 0.60	>*	0.38 \pm 0.12
Progeny recruits ($W_{R,j}$)					
1992	1.22 \pm 0.54	>	1.00 \pm 0.51	>	0.81 \pm 0.39
1998–1999	3.73 \pm 0.88	>>*	1.76 \pm 0.66	>>*	0.05 \pm 0.05

Shown are observed payoffs for relative fitness ($W = W_j/W_{\text{average}}$), which standardizes differences among years (siring; $W_{S,j}$; recruits; $W_{R,j}$; mean \pm SE) for $j =$ loner, recipient, and donor *bb* males, in years of high N_O (1992; $n = 6$ dyads, $n = 34$ loners) and low N_O (1998–1999; $n = 6$ dyads, $n = 22$ loners). Dyads of *bb* males are mutualistic ($W_{\text{recipient}} > W_{\text{donor}} > W_{\text{loner}}$) at low N_O (1998–1999) but altruistic ($W_{\text{recipient}} > W_{\text{loner}} > W_{\text{donor}}$) at high N_O (1992). Moreover, the interaction term for loner vs. donor \times RPS phase (high-low N_O) is significant for $W_{S,j}$ ($F_{1,64} = 10.50$, $P < 0.002$) and $W_{R,j}$ ($F_{1,64} = 9.61$, $P < 0.003$) (ln [$W + 1$]-transformed to normalize variance). Reversal in loner vs. donor rank order indicates a shift from mutualism to altruism. Significant differences in means (>) and interaction terms ($\swarrow \searrow$) are marked * ($P < 0.05$) or ** ($P < 0.01$). Wilcoxon tests compare loner and donor. Paired t tests compare recipient and donor.

Because few males survive more than one breeding season, future *bb* dyads form from progeny of recipients in altruistic phases of the RPS; few come from donors. Dyads are stabilized by high fitness of *b* alleles or genic selection but not destabilized by defection of egoists. Nonrandom settlement and cooperation of genetically similar *bb* males is, therefore, an evolutionary greenbeard that stabilizes dyads across generations. Because *bb* dyads have higher fitness than loner males, the greenbeard is best viewed as mutualistic in the long run. Periods of true altruism, restricted to generations in O phases of the RPS, are best viewed as “transient” altruism.

We have mapped key genetic factors for self-recognition of genetic similarity and a key signal locus, *OBY*. These loci express the three traits necessary for greenbeard behavior: signal, recognition, and donation (6). *OBY* color is a component of both greenbeard donation and signal, given relations with territoriality, stamina, testosterone, and aggression (23, 24). Thus, blue color is a signal for identifying other potential cooperators with similar territorial restraint, symmetry in stamina, and lower aggression relative to O males. Greenbeard recognition may also arise from self-referent phenotype matching (25). For example, self-referent signals like head-bob rate (26) or odor (27), used in individual recognition, could be the traits that are governed by color- and self-recognition loci (Fig. 1C) that stabilize *bb* cooperation. However, recombination of alternative alleles from Y and O genotypes among unlinked loci for the signal, self-recognition, and donation of *bb* cooperation will invariably generate many loner *bb* males that lack an intact greenbeard complex. Loner *bb* males may thus be deficient in one or more cooperative greenbeard traits, yielding low fitness relative to dyadic *bb* males. Our inferences on genomic architecture of *OBY* and self-recognition loci, based on the field pedigree, were greatly aided by the genetics of *Uta*, which harbors high levels of LD (Fig. 1) owing to chronic correlational selection that favors an intact signal-recognition complex in *bb* males, and alternative alleles that are favored in O and Y male strategies.

If Dawkins (6) had chosen another color in his hypothetical example of altruism, he might have presaged the bluebeard of cooperation in *Uta*. A salient difference between bluebeard recognition in *Uta* and Hamiltonian (5) supergenes envisioned by Dawkins (6) is the genome-wide nature of the *Uta* bluebeard. The *b* allele segregates at a key signal locus, but at least three other self-recognition and color-recognition factors (Fig. 1C) are linked to *OBY* signals by correlational selection on the benefits afforded by self-recognition and cooperation (15). Correlational selection exists because all signal-recognition donation components are required for *bb* cooperation, but such alleles are harmful for other morphs like O, which have aggressive behaviors that destabilize cooperation (15). Hamilton (5) did not consider the power of correlational selection in coupling suites of traits and driving coordinated evolution of the many loci required for complex behaviors like cooperation. Correlational selection that couples traits in one morph intensifies selection on other morphs in the next generation (15). Thus, social systems like the RPS cause adaptation to be self-reinforcing in a Red Queen cycle (13, 14, 28). The correlational selection that bundles components for signal, self-recognition, and self-attraction plays a profound role in building the genome-wide bluebeard of *Uta*. Correlational selection on recognition systems should be general for all forms of evolutionary cooperation (28).

Even in kin altruism, sharing a fraction of genes is not sufficient for donation; specific genes that allow recognition of kin, exclude cheaters, and coordinate donations are required. A proximate explanation for kin altruism is not that kin share a fractional number of genes; rather, kin altruists share key genes for signal, self-recognition, and donation behavior. Our view is that cooperators need to share alleles at three key loci for signal, recognition, and donation. Given that all three loci are required to secure the benefits of cooperation, correlational selection will build genetic

correlations among these loci. Overall genetic similarity of kin is thus a by-product of correlational selection on these loci rather than a cause of cooperation. Once key loci for cooperation exist, they cause genome-wide similarity to build by coopting more loci into this gene complex. Adding more loci allows for the complex to protect against erosion of linkages due to intragenomic conflict (10) and invasion by cheaters, as well as an added by-product of spreading benefits of cooperation across the genome.

One reason we may not find many examples of greenbeards is that we typically look for simple ones with few genes (7–12). Other examples with many shared genes are described as kin selection (29–31), even without a pedigree (32, 33). These cases may arise from greenbeard correlational selection acting on either kin or unrelated pairs. This process-based view of greenbeard correlational selection, which acts on many signal-recognition donation loci, provides a promising avenue for mapping genes that govern behavior in nature, as we have done in *Uta*. Our experiments on *Uta* also show that juvenile kin competition poses a severe constraint on evolution of kin cooperation in adults because dispersal is favored. Thus, juvenile kin competition enhances conditions for evolution of adult greenbeard cooperation. Conversely, novel social behaviors that ameliorate juvenile kin competition would enhance evolution of adult kin cooperation. For example, elaborate chambers constructed by social insects for juvenile development may have reduced sib competition, favoring evolution of adult kin altruism.

Do other species harbor cooperation arising from unlinked loci assembled in a coadapted complex of greenbeard signals, recognition, and donation? A fruitful place to look is in examples of kin altruism (29–31), which may harbor loci for signal, recognition, and donation behavior that are under correlational selection. In addition, the scarcity of examples of non-kin altruism may be because transient altruism is masked by long-term mutualism in studies that ignore temporal dynamics, or because mutualism would be expected to replace altruism over the evolutionary long run by means of a build-up of genes that stabilize cooperation. Cycles of altruism and mutualism may also be encouraged by RPS dynamics (28). The link between RPS cycles and altruism is highlighted by a bacterial RPS (34) played out among toxin-producing, toxin-resistant, and undefended clones. When crowded, some cells of toxic clones release toxins by autolysis. This self-sacrificial act reflects kin altruism that benefits the growing clone by destroying neighboring clones lacking resistance. In this sense, Eqs. 1–5 for greenbeard cycles, along with equations for other players of the RPS social system (16, 17), define a more general dynamic of alternative social strategies. We suggest that RPS strategies reflect more fundamental social strategies (35) of usurpation (O: badge of resource holding potential), cooperation (B: badge of cooperation), and deception (Y: sneaker) and that such social system dynamics are common in nature (reviewed in ref. 28). Perhaps altruism in other species can be maintained by similar frequency-dependent cycles of mutualism and altruism driven by these basic social strategies. In turn, such cycles of mutualism and altruism may provide the boost needed for successful spread of altruistic genes in early evolutionary stages of altruism.

Materials and Methods

Population Census. Maternal pedigrees are known with certainty: dams taken from nature lay eggs in the laboratory on first-to-third clutches (15–18). Dams are returned to territories between clutches. Marked progeny are randomly released with respect to parental territories (2–3 days hatching) on sib-randomized plots monitored since 1989. Progeny survival is censused at maturity. Adult *Uta* are restricted to patchy rock outcrops. The focal site is 400 m in radius. We search populations around this focal site (2-km radius). Emigration does not confound fitness (15, 18). Surviving progeny are unlikely to be missed; capture probability on a 3-week census is 0.97 (36). We census every 2–3 weeks to record color during the breeding season.

Territory Maps. Multiple passes are made over the site to map all male and female territories. For example, a total of $N = 1049, 801, 478,$ and 597 male locations were mapped in 1992, 1996, 1998, and 1999, respectively, yielding an average of 8.4, 13.8, 7.2, and 11.0 sightings per male (15). The minimum convex polygon that circumscribes locations defines a lizard's territory. Males with adjacent territorial edges are neighbors. Territory data (1990–2003) provide frequency of neighboring morphs ($N_B, N_O,$ and N_Y).

DNA Paternity. To build paternal links, we genotyped (on an ABI 377 from Applied Biosystems) nine microsatellites from extracted DNA by means of PCR (18). We genotyped 124, 56, 44, 40, 43, and 147 sires, 68, 96, 40, 35, 48, and 100 dams, and 458, 160, 212, 78, 103, and 284 progeny from 1992, 1996, 1998, 1999, 2001, and 2002, respectively. Paternity was assigned in KINSHIP (maximum likelihood and exclusionary criteria) (37). The delete-one jackknife method (38) was used to test for significance of regression (β_i) relating fitness to frequency of neighboring morphs.

Sibship-Intact Plots. We constructed four sibship-intact plots: three founded (14) in 1999 and one in 2000. Using the same methods described above, we released 910 male and 893 female hatchlings at sibship-intact sites (2001–2004). In 2002 we identified genetically similar *bb* dyads that formed by genotyping $n = 157$ males on the four sibship-intact plots [five most polymorphic (18) microsatellites].

Genetic Similarity. We used KINSHIP (37) to identify genetically similar neighbors (15) (e.g., relatedness comparable to sire–progeny or sib pairs; relatedness coefficient = 0.5). We searched all pairwise cases of sires in a neighborhood. Maximum likelihood (18, 37) was used to establish which males met this “kin” criteria ($P \leq 0.01$) relative to all other males rejected as kin ($P > 0.01$). Neighboring males could be sibling or sire–progeny pairs, reflecting high genetic similarity; however, none were kin (e.g., sibs, half-sibs, cousins, and sires) based on the maternal pedigree (18).

Male Association Tests. Across all possible pairwise comparisons of *bb* males (279 pairs) residing in the same population, we found that

15 pairs met the maximum likelihood criterion of genetic similarity (see *Genetic Similarity*), or 5.4% of *bb* pairs. Eighty-two *bb* pairs were actual neighbors; thus, we expect 4.4 genetically similar *bb* pairs by chance alone to be neighbors. A similar test was used on sibship-intact plots, but we also pooled males into a non-*bb* class (see *Dyad Formation and Kin Philopatry*).

Linkage Mapping. We built a primary linkage map of nine microsatellites (1992 pedigree: 48 families, $n = 368$) using CRIMAP 2.4 (39). We mapped genetic variation and coded for color alleles (14) and self-recognition (1998–1999 pedigree: 25 families, 105 individuals). Significant transmission LD (CRIMAP 2.4, logarithm of odds ≥ 3.0) (40) between genetic variation in *OBY* color alleles and microsatellite marker loci indicated cosegregation on the same linkage. Markers with logarithm of odds < 3.0 recombined at 0.5 (e.g., 100 cM). We mapped self-recognition behavior, coded as 0 for males not forming dyads with genetically similar males, 1 for dyadic males with genetically similar neighbors with one *b* allele, and 2 for dyadic males with genetically similar neighbors with at least two *b* alleles. Dyadic behavior is not restricted to *bb* dyads, but also drives female settlement. The pedigree indicates that few males mated with female kin (15), so “female–male dyadic” behavior may also be due to shared genes for recognition. Females were scored as above, 0, 1, and 2+, based on number of genetically similar mates that also had *b* alleles.

Rock Pile Experiment. We manipulated the thermal environment by building 75 rock piles (0.5–1.0 m), which are attractive to both sexes (20, 22). Rock piles were 0.5–1.0 m in height, composed of 10–40 boulders, and spaced at 35.5 ± 3.1 m (SE). Adults ($n = 244$ males) recruited naturally to rock piles ($\approx 80\%$), and we also seeded hatchlings from laboratory crosses (20%) (20, 22).

We thank D. Friedman, G. Pogson, A. Corl, S. Kuchta, J. Vogelzang, and B. Lyon for discussions and the Arbielbiedee, Hultgren, Stadler, and Schrimp families for access to land. This work was supported by the National Science Foundation, a National Geographic Grant, and Centre National de la Recherche Scientifique fellowships (to B.S.); a France–Berkeley grant (to B.S. and M.E.H.); a National Science Foundation Doctoral Dissertation Improvement Grant (to R.C.); and the Natural Sciences and Engineering Research Council (Canada) (A.G.M.).

- Koella, J. C. (2000) *Proc. R. Soc. London B* **267**, 1979–1985.
- Axelrod, R., Hammond, R. A. & Grafen, A. (2004) *Evolution (Lawrence, Kans.)* **58**, 1833–1838.
- Komdeur, J. & Hatchwell, B. J. (1999) *Trends Ecol. Evol.* **14**, 237–241.
- Frank, S. A. (2003) *Evolution (Lawrence, Kans.)* **57**, 693–705.
- Hamilton, W. D. (1964) *J. Theor. Biol.* **7**, 1–52.
- Dawkins, R. (1976) *The Selfish Gene* (Oxford Univ. Press, Oxford).
- Keller, L. & Ross, K. G. (1998) *Nature* **394**, 573–575.
- Queller, D. C., Ponte, E., Bozzaro, S. & Strassmann, J. E. (2003) *Science* **299**, 105–106.
- Foster, K. R., Shaulsky, G., Strassmann, J. E., Queller, D. C. & Thompson, C. R. L. (2004) *Nature* **431**, 693–696.
- Haig, D. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 6547–6551.
- Summers, K. & Crespi, B. (2005) *Proc. R. Soc. London B* **272**, 643–649.
- Moore, H., Dvorakova, K., Jenkins, N. & Breed, W. (2002) *Nature* **418**, 174–177.
- Sinervo, B. & Svensson, E. (2002) *Heredity* **89**, 329–338.
- Sinervo, B., Bleay, C. & Adamopoulou, C. (2001) *Evolution (Lawrence, Kans.)* **55**, 2040–2052.
- Sinervo, B. & Clobert, J. (2003) *Science* **300**, 1949–1951.
- Sinervo, B. (2001) *Genetica* **112**, 417–434.
- Sinervo, B. & Lively, C. M. (1996) *Nature* **380**, 240–243.
- Zamudio, K. & Sinervo, B. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 14427–14442.
- Sinervo, B. (2001) in *Model Systems in Behavioral Ecology*, ed. Dugatkin, L. (Princeton Univ. Press, Princeton), pp. 93–106.
- Calsbeek, R. G. & Sinervo, B. (2002) *Proc. Natl. Acad. Sci. USA* **99**, 14897–14902.
- Calsbeek, R. & Sinervo, B. (2002) *Oecologia* **138**, 468–477.
- Calsbeek, R. G. & Sinervo, B. (2002) *J. Anim. Ecol.* **71**, 513–523.
- Calsbeek, R., Alonzo, S. H., Zamudio, K. & Sinervo, B. (2002) *Proc. R. Soc. London B* **269**, 157–164.
- Sinervo, B., Miles, D. B., Frankino, W. A., Klukowski, M. & DeNardo, D. F. (2000) *Horm. Behav.* **38**, 222–233.
- Hauber, M. E. & Sherman, P. W. (2001) *Trends Neurosci.* **24**, 609–616.
- Brandt, Y. & Allen, J. R. (2004) *Behav. Ecol. Sociobiol.* **55**, 257–265.
- Lena, J.-P., de Fraipont, M. & Clobert, J. (2000) *Ecol. Lett.* **3**, 300–318.
- Sinervo, B. & Calsbeek, R. (2006) *Annu. Rev. Ecol. Syst.*, in press.
- Shorey, L., Piartney, S., Stone, J. & Høglund, J. (2000) *Nature* **408**, 352–353.
- Baglione, V., Canestrari, D., Marcos, J. M. & Ekman, J. (2003) *Science* **300**, 1947–1949.
- Krakauer, A. H. (2005) *Nature* **434**, 69–71.
- Dickinson, J. L. & Koenig, W. D. (2003) *Science* **300**, 1887–1889.
- Grosberg, R. K. & Hart, M. W. (2000) *Science* **289**, 2111–2114.
- Kerr, B., Riley, M. A., Feldman, M. W. & Bohannan, B. J. M. (2002) *Nature* **418**, 171–174.
- Hochberg, M., Sinervo, B. & Brown, S. (2003) *Evolution (Lawrence, Kans.)* **57**, 154–158.
- Sinervo, B., Calsbeek, R., Comendant, T., Both, C., Adamopoulou C. & Clobert, J. (2006) *Am. Nat.*, in press.
- Goodnight, K. F. & Queller, D. C. (1999) *Mol. Ecol.* **8**, 1231–1234.
- Mitchell-Olds, T. & Shaw, R. (1987) *Evolution (Lawrence, Kans.)* **41**, 1149–1161.
- Lander, E. S. & Green, P. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2363–2367.
- Ott, J. (1999) *Analysis of Human Genetic Linkage* (Johns Hopkins Univ. Press, Baltimore).