THE EVOLUTION AND MAINTENANCE OF COOPERATION IN NATURAL AND ARTIFICIAL POPULATIONS

By

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ABSTRACT

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Cooperative behaviors, where an individual benefits another at its own expense, pose a special problem for biologists. Although populations of cooperative individuals have higher average fitness than populations of uncooperative individuals, cooperative populations are open to invasion by uncooperative individuals. Proposed mechanisms for how cooperation can resist invasion by uncooperative individuals fall into four broad categories, kin selection, group selection, reciprocal altruism and by-product mutualism. The primary goals of my research was to: 1) determine the mechanism responsible for the maintenance of two cooperative behaviors in red-winged blackbirds; 2) explore the role extra-pair paternity plays in cooperation 3) use simulations to show how cooperative behaviors become established in a population of uncooperative individuals.

Reduced aggression among territorial males is potentially a form of cooperation because individuals are tempted to cheat by unilaterally expanding their territory or by seeking extra-pair copulations on their neighbor's territory. I used simulated defections to determine if reduced aggression was a form of reciprocal altruism. I used paternity analysis coupled with behavioral observations of territorial behavior to estimate the frequency of cheating and to

determine if males respond to cuckoldry by their neighbors. The simulated defections showed that males responded to simulated defections by increasing territorial aggression towards the neighbor, a result indicative of reciprocal altruism. My results also show that males are more aggressive towards males that have successfully cuckolded them. Additional evidence suggests that males may be able to assess a neighbor's ability to cuckold rather than directly detect cuckoldry.

I also used simulated defections combined with paternity analysis of nestlings to simultaneously test between reciprocal altruism and by-product mutualism. Previous studies have provided evidence for either hypothesis although no study has tested for both in the same population. Males in the population I studies appear to cooperative nest defense primarily as a form of reciprocal altruism. There was no evidence of by-product mutualism in the form of males defending nests on other territories in which they had obtained extra-pair fertilizations.

Simulations using genetic algorithms show that population structure enhances the evolution of cooperation from an uncooperative population. Populations composed of small subpopulations achieved higher rates of cooperation than populations composed of large subpopulations. Additionally, population structure influenced the strategies that evolved. The common strategies in the smallest populations cooperated almost unconditionally. The most common strategies in lightly larger populations, however, were similar to Tit-For-Tat.

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CHAPTER 1:

MALE REPRODUCTIVE SUCCESS IN RED-WINGED BLACKBIRDS: LACK OF TRADE-OFF DUE TO FEMALE CHOICE

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ABSTRACT

Molecular analysis of paternity has demonstrated that realized male reproductive success may be substantially different from on-territory nesting success. The contribution of extra-pair paternity to male reproductive success, however, is seldom obvious. Males that obtain large harems or productive territories may suffer reduced on-territory reproductive success because they must invest more time and energy into defense and maintenance. Males who invest less in acquisition of territory and defense may seek more extra-pair copulations. Alternatively, males with good territories and large harems may be more attractive to females because they can provide extra-pair young with either better resources or better genes. We used analysis of microsatellite loci to genetically determine the mating success of males in relation to their observed mating success. We looked for evidence that successful males are physical different from those who are not. We also studied the effect that male density had on the reproductive success of males. Males that were successful in fledging young on their own territory also obtained the most EPFs and were cuckolded less. We found that size (estimated using PCA analysis) and wing chord correlated positively with number of young fledged on the territory, nests on the territory and number of EPCs achieved by the male but not total reproductive success. Males with neighbors on the same pond were cuckolded more than males who held an entire pond as a territory. We found little evidence for a trade-off in male reproductive strategies. The effects of female choice likely over shadow any trade-off faced by male red-winged blackbirds.

INTRODUCTION

With recent advancements in molecular paternity analysis it has become apparent that observed reproductive success and realized reproductive success may not be the same (Gibbs et al., 1990; Petrie and Kempenaers, 1998; Weatherhead and Boag, 1997). Off-territory reproduction, in the form of extra-pair fertilizations (EPFs), may either decrease or augment a male's on-territory reproductive success. Determining effect of extra-pair paternity on reproductive success is important because estimating total male reproductive success by using territory production may not be accurate if extra-pair paternity is frequent (Gibbs et al., 1990). Additionally, extra-pair paternity can alter the degree of sexual selection by increasing or decreasing the variance in reproductive success among males (Weatherhead and Boag, 1997).

Extra-pair paternity might decrease a male's reproduction if there is a trade-off between reproduction on a male's own territory and ability to achieve extra-pair copulations (EPCs). Males that are able to obtain productive territories and large harems may have to spend more time protecting their territory and harem against intruding males. Males that are successful at reproducing on their own territory might therefore be less able to gain extra-pair copulations and might also suffer more from cuckoldry on their own territory (Hasselquist and Sherman, 2001; Westneat, 1993b). Additionally, males that invest more time into seeking EPCs may suffer from higher cuckoldry, reduced on territory mating success or reduced parental care (Sherman and Morton, 1988; Westneat, 1988; Westneat, 1993a)

In contrast, there are also reasons to expect positive relationships between a male on-territory reproductive success and off-territory reproductive success. Extra-pair paternity would increase with on-territory reproductive success if females choose males that were able to establish territories on productive habitat (Hasselquist and Sherman, 2001). In this case, females control the frequency of extra-pair fertilization in their young (Gray, 1996). Alternatively, a positive correlation might also result even if males control extra-pair paternity. If the variance in male quality is sufficiently high, low quality males could do relatively worse even though they suffer proportionally less from cuckoldry. This would result in a positive correlation when all males are considered together (Stearns, 1989; Stearns, 1992).

Red-winged blackbirds are an ideal species to study such questions. This species exhibits a high degree of variability in reproductive success, due primarily to variation in habitat (Turner and McCarty, 1998; Weatherhead and Robertson, 1977). In addition, they have been shown to engage in significant levels of extra-pair copulation (Gray, 1997a; Gray, 1997b; Moller, 2000; Weatherhead et al., 1994).

Female red-winged blackbirds have been shown to seek extra-pair copulations and control the level of extra-pair paternity in their nest (Gray, 1996; Gray, 1997a). However, the relationship between a male on-territory and off-territory reproductive success is unclear. Weatherhead (1997) found that male off-territory reproduction was positively correlated with on-territory reproduction and negatively correlated with the level of cuckoldry on a male's territory. Gibbs (1990) on the other hand, found that while off-territory reproduction correlated negatively with cuckoldry there was no relationship between on-territory reproduction and off-territory reproduction.

In this study, we examine the relationship between extra-pair paternity, on territory paternity and fledging success within a territory to test for trade-offs in population a population with almost no nest predation. This allows us to examine the relationship between off-territory and on-territory reproductive success without the potentially confounding affects of cooperative nest defense (Gray, 1997b; Weatherhead et al., 1994). We also look for evidence that male quality, measured by size, is associated with either a male's success at raising fledglings or a male's ability to gain extra-pair fertilizations (EPFs) or the number of young produced off territory.

METHODS

This study was conducted at the Kellogg Biological Station Experimental Pond Facility, Hickory Corners, Michigan (42° 24' N, 85° 24' W) over four years, 1996-1998. We only performed paternity analysis in 1998. Each of 18 ponds was approximately 30 m in diameter and 3 m deep. The ponds were arranged in three rows of six. Within rows, ponds were approximately 5 meters apart, rows were spaced approximately 10 meters apart (Figure 1). The margin of each pond was densely vegetated, predominately with cattails, *Typha latifolia*.

Although the ponds were constructed primarily for aquatic research, they were consistently colonized by red-winged blackbirds. During this study, one to four males settled each pond with each male holding a harem of one to six females. A chain link fence surrounded the facility keeping out most predators; therefore nesting success was very high (near 100%).

We marked every territorial male with a unique combination of three colored leg bands and a numbered aluminum. We then measured weight, culmen length, tarsus length and wing chord of each male and determined its age using plumage criteria (Pyle, 1997).

We mapped each male's territory using behavioral criteria (Beletsky and Orians, 1987). The entire pond was defined as a male's territory when he was the only resident male on that pond. On ponds with more than one male, we defined territorial boundaries by where two neighboring males counter-sang (both males within 10 m of each other and singing in alternate order), the limit of the resident's movement, or the center of overlap between two neighboring males' movement. We estimated male harem size as the maximum number of simultaneously active nests.

We searched for nests daily. We located most nests as they were being built and found no nests later than a week after completion, so we are reasonably certain we found all nests. We marked the location of each nest with flagging tape at the edge of the pond and we monitored nests every 4 to 8 days until the fate was determined. Although females often raise multiple broods in this population, we incorporated data from only the first brood to limit pseudo-replication and differences among broods.

We obtained 50-80 ml of blood from all territorial males and many females. We also took approximately 0.5 ml of blood from nestling when they were 8 days old. Blood was drawn from the brachial vein, and immediately placed in 800 ml of "Queen's" lysis buffer (Seutin et al., 1991).

DNA was extracted from the blood samples using Proteinase K digestion followed by extraction in 7.5M NH_4A_0C and precipitation in isopropyl alcohol. The DNA was washed once more in 70% ethyl alcohol.

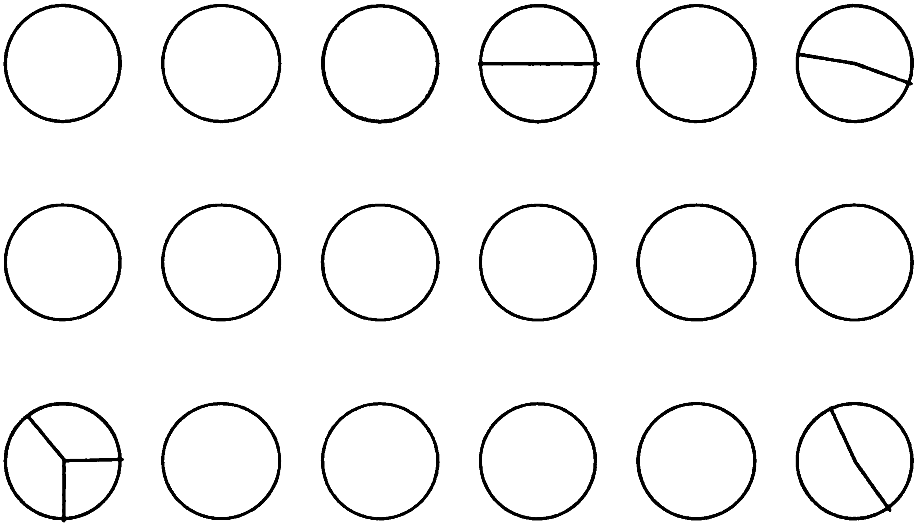

Figure 1. The spatial arrangement of the ponds used in this study. Ponds were 30 m in diameter and approximately 3 m maximum depth. Ponds on the same row were approximately 5 meters apart while rows were approximately 10 meters apart. Lines across ponds show territory sizes on ponds with multiple territories. Ponds without and divisions held a single territory.

We determined paternity of nestlings using six microsatellite loci. Four of the loci (Qm 5, Qm 10, Qm 21, Qm 31) were developed for great-tailed grackles (*Quiscalus mexicanus*) (Gibbs et al., 1997), Dpμ 16 for yellow warblers (*Dendroica petechia*) (Dawson et al., 1997) and Maμ 10 for brown-headed cowbirds (*Molothrus ater*) (Gibbs et al., 1997).

We assayed genetic variation at these loci using PCR amplification in 25 μL reaction volumes. Two different reaction conditions were required for the six loci. Qm 10, Dpμ 16 and Maμ 10 were amplified using 250 ng of template DNA, 2 pmol of each primer (fluorescently labeled forward primer), 500 μM dNTPs, and .75 U Taq polymerase. Qm 5, Qm 21 and Qm 31 were amplified using 125 ng template DNA, 1.25 pmol of each primer (fluorescently labeled forward primer), 625 μM dNTPs and .75 U Taq polymerase. All reactions were performed in buffer containing 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 30mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01 Triton X-100. The PCR product was run on 6% polyacrylamide gels and visualized using an FMBIO gel scanner. We assigned genotypes to all individuals based on internal lane size standards and individuals of known genotype run concurrently o the gel. All gels were scored by hand by both authors and verified using FMBIO image analysis software.

We included only territorial males as potential fathers, since previous work has shown that floating males rarely gain fertilizations (Gibbs et al., 1990). We determined the paternity of all offspring employing either exclusion criteria or maximum likelihood methods using Cervus (Marshall et al., 1998). Using the six loci described above, the probability of assigning the wrong father to an offspring was 0.7%. We were able to establish paternity for all but two of 252 offspring for which we obtained DNA. For 217 of the young, there was only one non-excluded candidate parent. Using maximum likelihood estimates of paternity, we were able to assign paternity to most of the remaining offspring with 95% confidence level (26 offspring), but we also included assignments at the relaxed confidence of 80% (7 offspring).

All correlations shown are Pearson's correlations. We arcsine transformed proportional data (i.e. proportion of young cuckolded). All significance values are two tailed.

RESULTS

The overall rate of extra-pair paternity in 1998 was 36.2% of 252 offspring and 52.4% of 93 nests for which we obtained DNA. Only 2 of 252 offspring appeared to be sired from males not on our study site. Among the extra-pair offspring, a neighbor was the genetic father 88% of the time.

Each male fledged an average of 13.5 (±5.50 SD) offspring on its territory. The average total reproductive success for each male, based on paternity analysis was 11 (±6.15 SD) offspring. On average each male sired 4.1 (±3.57 SD) extra-pair offspring. The overall effect of extra-pair paternity was to slightly reduce each male's reproductive success while causing a moderate increase in the variance in reproductive success.

Ponds were the primary determinant of nest success in our population. The return rate by males onto the same pond was 27% over the four years of the study. However, ponds were consistent among years in number of nestlings fledged (Kendalls tau, $\tau_{17} = 0.82$, $P < 0.001$) as well as higher probability of nest success (Kendall's tau, $\tau_{17} = 0.73$, $P < 0.001$).

There was a positive relationship the number of young fledged and number of EPFs obtained by that male on other territories ($r_{19} = 0.58$, $P < 0.01$; Figure 2a). However, there was no relationship between the number of nests on a territory and number of EPFs obtained by that male ($r_{19} = -0.13$, N.S.; Figure 2b). The highly successful male in Figure 1a did not appreciably alter the correlation when excluded from the analysis ($r_{19} = .48$, $P < 0.05$). Number of young fledged on a territory was negatively correlated with proportion of nests cuckolded on that territory ($r_{19} = -0.70$, $P < 0.001$; Figure 3a). However, there was no association between number of nests on a territory and nests that were cuckolded on that territory ($r_{19} = -0.36$, N.S.; Figure 3b). Total male reproductive success (total number of fertilizations obtained on the study site) was positively correlated with both the number of young fledged on the territory ($r_{19} = 0.92$, $P < 0.001$; Figure 4a) and number of EPFs obtained ($r_{19} = 70$, $P < 0.0001$; Figure

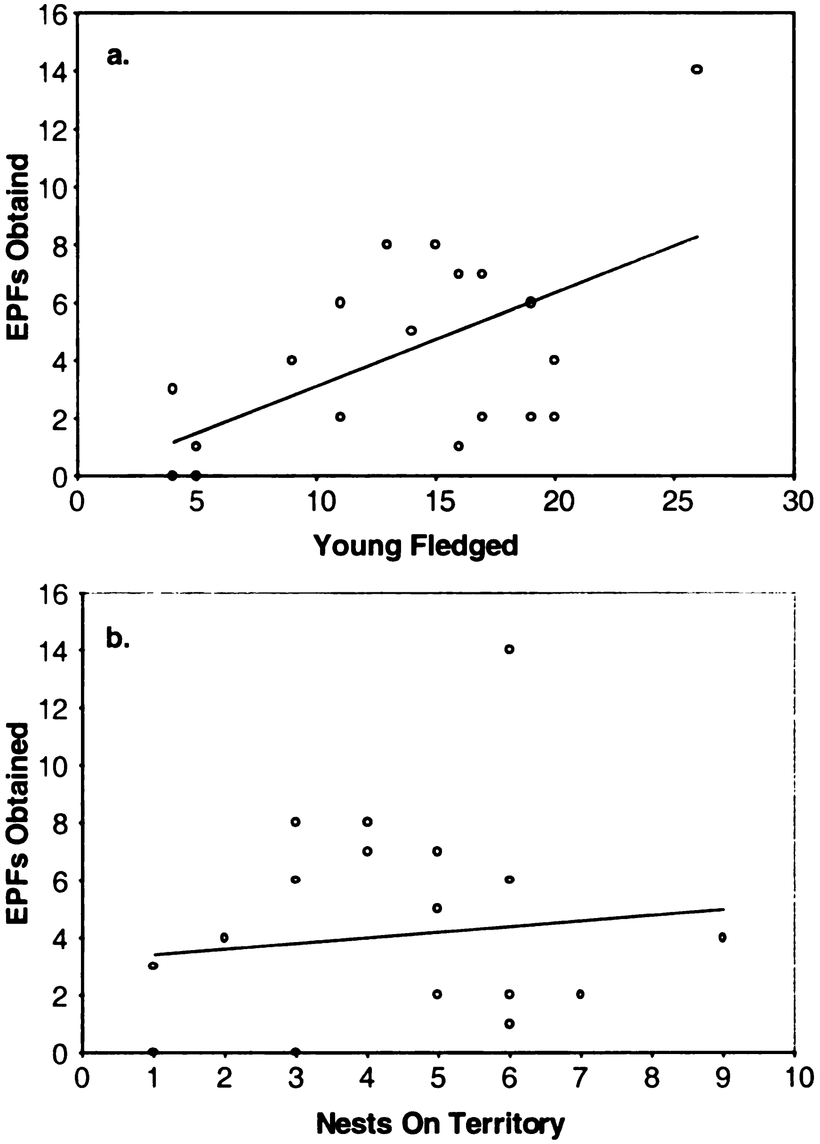

Figure 2. The association between the number of EPFs obtained by a male and **a.** the number of young fledged from a male's territory or **b.** number of nests on a male's territory. N = 19.

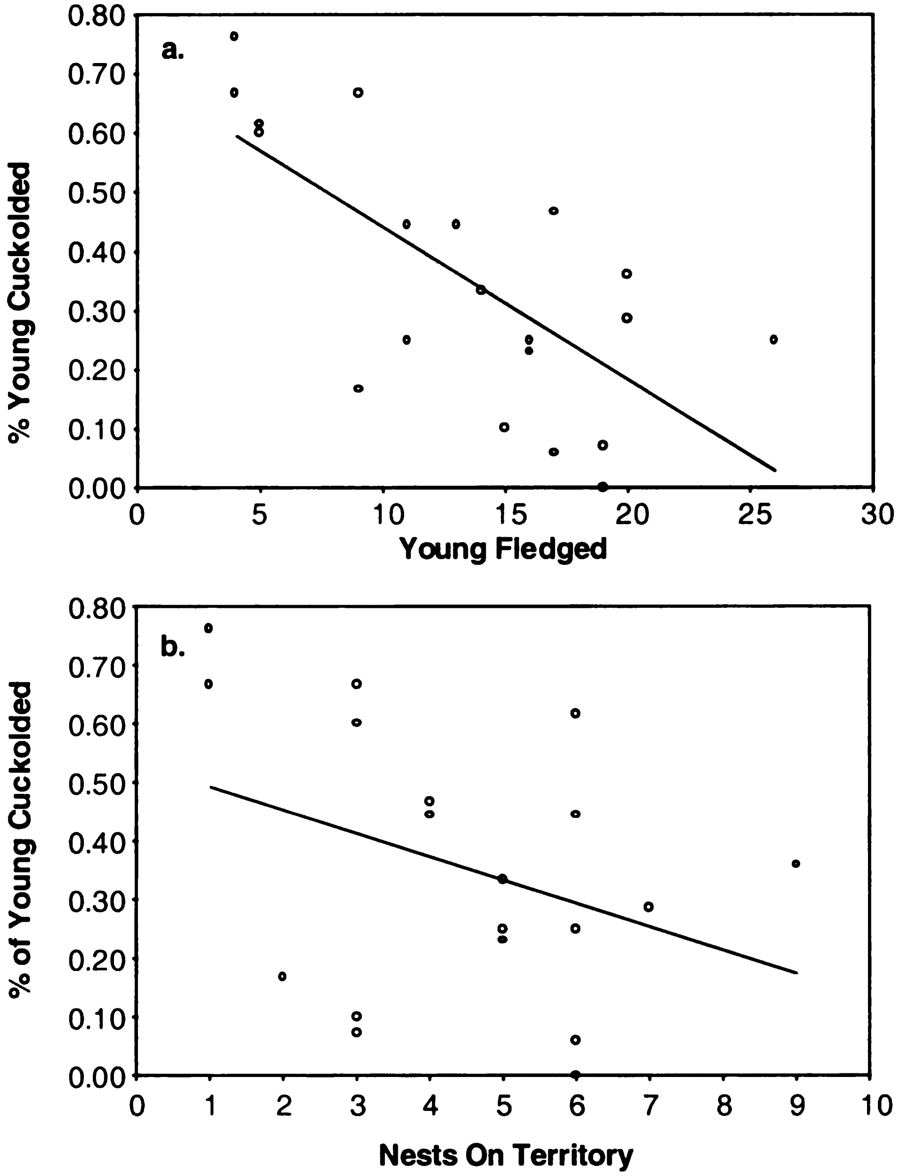

Figure 3. The association between the percent of a male's young that were cuckolded and **a.** the number of young fledged from a male's territory and **b.** number of nests on a male's territory. N = 19.

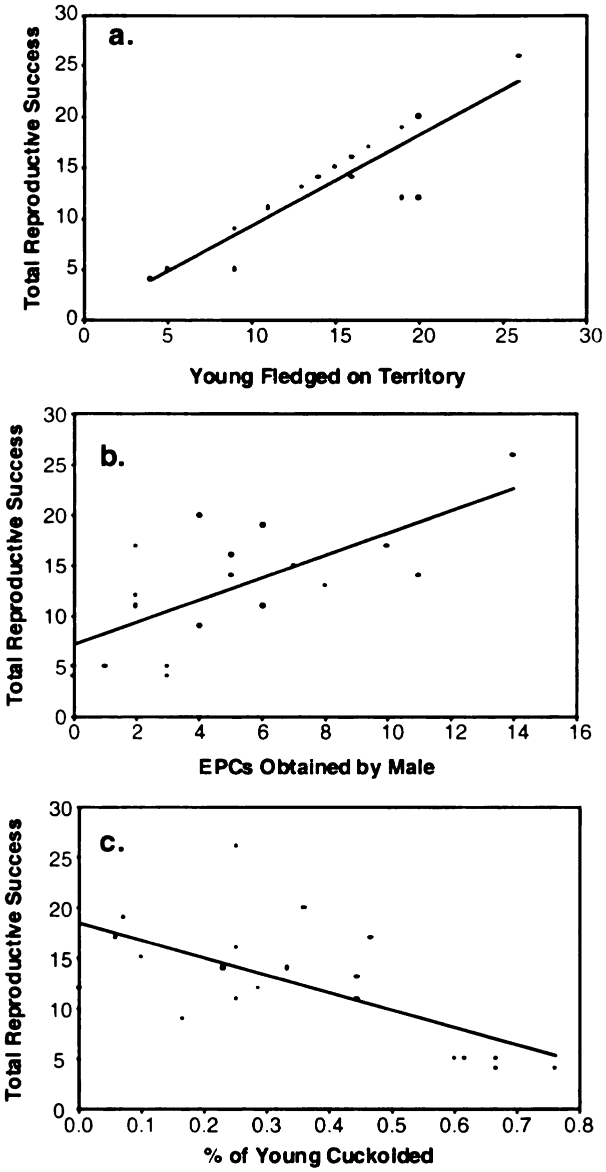

Figure 4. The relationship between total male reproductive success (number of fertilizations over the entire site) and **a.** Young fledged on a male's territory; **b.** EPCs obtained by a male and **c.** proportion of young on a male's that was cuckolded. N = 19 males.

4b). Male reproductive success was negatively correlated with proportion of offspring cuckolded on the territory ($r_{19} = -0.67$, $P < 0.005$; Figure 4c).

We performed a PCA analysis on the size data from every male caught in 1998. The first principle component accounted for 45.2% of the variance and the component variables all loaded such that an increase in PC1 was associated with an increase in size. We therefore used PC1 as a measure of size. PC2 accounted for 24.5% of the variation and was associated with culmen size relative to weight (Table 1).

Male size (PC1) correlated positively and significantly with the number of EPFs obtained, number of young fledged and total reproductive success. However, male size did not correlate with proportion of young cuckolded. Wing cord was the only component variable to correlate significantly with any measure of reproductive success. PC2 did not correlate significantly with any measure of reproductive success (Table 2).

Males with neighbors on the same pond raised fewer young to fledging ($r_{19} = -0.513$, $P < 0.025$) and had fewer nests ($r_{19} = -0.571$, $P < 0.01$). The proportion of young cuckolded on a territory increased with the number of territories on a pond ($r_{19} = 0.52$, $P < 0.25$). However, males with more neighbors did not achieve more EPFs ($r_{19} = -0.32$, N.S.).

Table 1. The results of a PCA analysis performed on four physical traits measured on red-winged blackbirds. PC1 is associated with size and PC2 is associated with culmen length relative to weight.

	PC1	**PC2**
Weight	0.68	0.61
Wing Cord	0.84	0.08
Tarsus	0.62	-0.14
Culmen	0.51	-0.77
% Variance Explained	45.19	24.49

Table 2. Correlation coefficients between a male physical traits and measures of reproductive success.

	PC1	PC2	Wing	Weight	Tarsus	Culmen
EPFs Obtained	0.54 **	-0.22	0.23	0.07	0.25	0.06
Young Fledged	0.55 **	-0.10	0.45 *	0.22	0.19	-0.07
Nests on Territory	0.48 *	0.12	0.62 ***	0.37	0.10	0.12
Total Reproductive Success	0.46 *	-0.11	-0.17	0.19	0.30	-0.19
% Young Cuckolded	0.16	0.07	-0.12	0.13	0.25	-0.05

* P < 0.05; ** P < 0.025; *** P < 0.001

DISCUSSION

We found that there was little evidence for trade-offs between on-territory reproduction and off-territory reproduction in male red-winged blackbirds. Males who fledged the most young on their own territory were also better at both obtaining their own EPFs and preventing their own young from being cuckolded.

We also found that male size correlated with number of nests on the territory, young fledged and the number of EPCs obtained. However, male size did not correlate with cuckoldry on the male's own territory.

A trade-off between within territory reproductive success and extra-pair paternity is expected because the more resources an individual invests into one mode of reproduction, the less it should be able to invest in others (Stearns, 1989; Stearns, 1992). It seems reasonable therefore to expect that males who invest time and energy into obtaining and defending high quality territories might be less successful in gaining EPFs. Similarly, males who find themselves on low quality territories might invest more energy into seeking EPFs (Hasselquist and Sherman, 2001).

Despite this expectation, we found no direct evidence of a trade-off. Males who fledged the most young on their own territory were also most successful in gaining EPFs. Additionally, males that fledged the most young suffered proportionately less from cuckoldry. Weatherhead & Boag (1997) found similar results in a Washington population of red-winged blackbirds. Weatherhead & Boag also found positive correlations between current and future reproduction as well. There are two possible explanations for these positive correlations.

First, a trade-off can be masked by large variation in quality among individuals (Stearns, 1989; Stearns, 1992). Trade-offs may be occurring, but they remain hidden because high quality males from high quality territories always outperform males from low quality territories. A correlation among all males would therefore yield a positive relationship between territory quality and measures, such proportion of young cuckolded. We have some evidence that this might occur in our population. We found considerable variation in reproductive success among males, possibly enough to account for such an effect. We also found that the proportion of young cuckolded on territories with neighbors on the same pond was higher than on ponds with a single territory. Since ponds with multiple territories were also the most productive, the overall effect was to mask the trade-off and show a positive correlation.

The other possible explanation for the lack of a trade-off is female choice. Nest success in this population is largely dependent on the pond on which the territory is located (Eckert and Weatherhead, 1987; Weatherhead and Robertson, 1977). We found a high concordance among years in pond productivity while at the same time only 25% of the males returned to a territory. Females therefore appear to settle on the most productive ponds. Females that are unable to settle on a high quality territory may “make the best of a bad situation” by seeking EPCs with males on high quality ponds. Females are able to gain greater foraging access on these high quality territories and may benefit from additional nest defense from other territorial males (Gray, 1997b).

Females may also benefit genetically from EPCs if they mate mostly with males on high quality territories. We found evidence that larger males are more likely to obtain higher quality territories than smaller males and larger males obtain more EPFs than smaller males. If size were heritable then the young would be larger and more likely to obtain high quality territories in the future. These results corroborate Gray’s (1997a) results that females benefit genetically by seeking EPCs.

Male Quality, Territory Settlement and Reproductive Success

Male quality correlated significantly with territory productivity and several measure of reproductive success. We found a positive relationship between male size and pond productivity. Larger males fledged more young on their territory and had more nests on their territory. Previous studies suggest that male-male competition determines settlement pattern and females choose nesting sites based on-territory quality (Picman, 1987; Searcy and Yasukawa, 1995). It therefore appears that larger males are better able to obtain good territories and females choose mates based on their territory. Other studies have also found that large males enjoy higher reproductive success on their own territory (Rohwer et al., 1996; Searcy, 1979). Size would therefore appear to influence female choice indirectly through territory settlement. Large males are better able to obtain high quality territories and females then choose to settle on high quality territories (Figure 5).

In addition to higher on-territory reproductive success, larger males on our study site also achieved more EPCs than smaller males. This suggests that not only are larger males able to settle on better territories but they are also able to gain copulations with females, both on their territory and off. This result suggests that females not only seek EPCs with males to gain material benefits (Gray, 1997b; Weatherhead et al., 1994) but also obtain genetic benefits. If females mate with large males then the young are likely to be larger as well. The offspring would therefore be more likely to obtain larger territories and more matings. Female choice therefore appears to operate directly on male reproductive success by increasing a male’s off-territory reproduction (Figure 5). This is consistent with the “good genes” hypothesis (Kempenaers et al., 1997; Moller, 2000) and corroborates other studies that suggest that females seek EPCs in order to gain genetic benefits (Gray, 1997a).

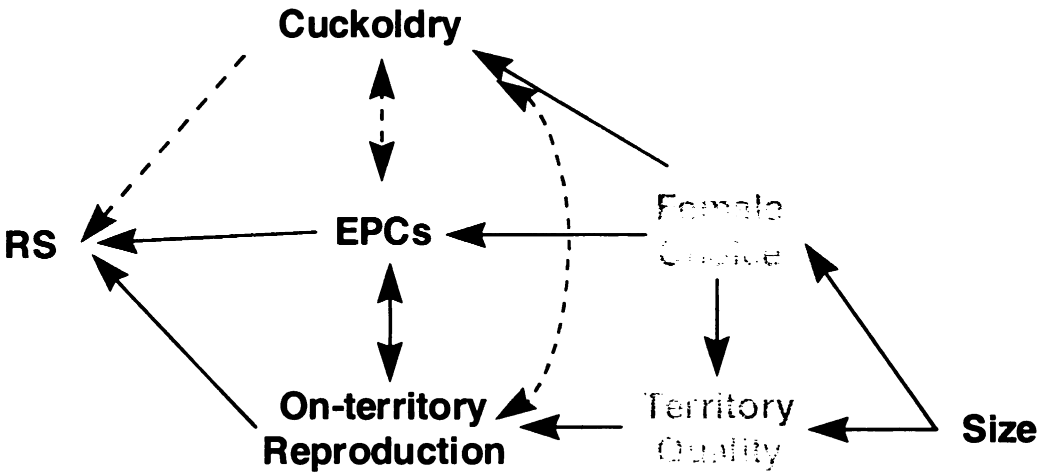

Figure 5. Proposed relationship between size, EPCs, on-territory reproduction, cuckoldry and reproductive success. Words in gray represent unmeasured variables that thought to play a role in male reproductive success. Lines with one arrow show relationships where significant correlations were found and causality is inferred. Double headed arrows show relationships where causality cannot be inferred. Solid lines indicate positive relationships, dashed lines represent negative relationships.

We found little evidence that there is a trade-off between on-territory and off-territory reproduction. Large males fledged the most offspring from their territories, a smaller proportion of their young were cuckolded and they were able to obtain more EPFs. This is consistent with other studies that show positive relationships between other aspects of male reproductive success (Hasselquist and Sherman, 2001; Weatherhead and Boag, 1997). We also show that size correlates with all aspects of male reproductive success except proportion of young cuckolded. Taken together, this study supports the conclusions that females choose territories based on territory quality but seek EPCs based on male quality.

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CHAPTER 2:

REDUCED TERRITORIAL AGGRESSION AMONG "DEAR ENEMIES" IN RED-WINGED BLACKBIRDS: TIT-FOR-TAT BEHAVIOR WITH CRYPTIC DEFECTORS

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ABSTRACT

While competing for similar resources, neighboring territorial males may benefit by cooperating to achieve common goals. Reduced aggression among territorial neighbors is one form of cooperation where individuals respect one another's territorial boundaries. Each neighbor benefits by being able to reduce the time and energy spend defending its boundaries. However, if defections (cheating) are cryptic the frequency of defection might be higher than expected when defection is more conspicuous. We used simulated territorial invasions to test for retaliation by red-winged blackbirds in response to defection by neighbors. We also used analysis of microsatellite regions of DNA to determine

the degree of cheating, in the form of cuckoldry, that might occur between neighbors and to determine if males increased their aggression towards cuckolding neighbors. We found that males increased aggression towards neighbors when we simulated intrusions by that neighbor, but not when we simulated intrusions by strange males. The over all rate of cuckoldry was relatively high suggesting a high rate of defection among males. Males were more aggressive towards neighbors that had sired offspring in their nest, but aggression was also positively correlated with a neighbor's overall ability to gain extra-pair copulations. The results of our study support the hypothesis that reduced aggression among "dear enemies" is a form of reciprocal altruism using TFT like strategies. We argue that males do not respond directly to cheating by neighbors but rather react more aggressively towards males that are "attractive" to females.

INTRODUCTION

In territorial species where males hold closely adjacent territories, neighbors may often be in a simultaneously competitive and cooperative relationship. While competing for resources such as territory, mates or food, neighboring males also share common enemies such as nest predators and other rival males. They might therefore benefit by working together to accomplish shared goals. Neighboring males locked in this simultaneously competitive and cooperative relationship are known as “dear enemies” (Fisher, 1954; Getty, 1987).

Consistent with this hypothesis is the finding that territorial neighbors often behave less aggressively with each other than with strangers at their territorial boundary (Brindley, 1991; Stoddard et al., 1991; Ydenberg et al., 1988). By reducing aggression with each other, “dear enemies” may benefit by reducing the amount of time and energy each male spends guarding its territory. However, there is a temptation for each neighbor to cheat by either unilaterally expanding its territory or sneaking copulations with its neighbor’s mates and realizing at least a short term gain (Getty, 1987).

Situations where cooperation is mutually beneficial but there is still a temptation to cheat are often modeled in game theory using the Prisoner’s Dilemma (PD). The PD was first developed in the economic and social sciences by Von

Neuman (1953). The PD was subsequently adapted for use in evolutionary theory by Axelrod and Hamilton (1981) who developed the model using the framework of evolutionarily stable strategies (Maynard Smith, 1982). In the PD, two players may either cooperate or defect. If both players cooperate, they both receive a high payoff (R) while mutual defection rewards both players with a lower payoff (P). If one player defects while the other cooperates the defecting player receives the highest possible payoff (T) while the cooperating player receives the lowest possible payoff (S). In other words, the payoff structure must conform to the inequality ($T>R>P>S$) (Von Neuman and Morgenstein, 1953). A second condition ($T + S < 2R$) is frequently imposed as well. This condition ensures that individuals cannot do better than R by alternating between the T and S payoffs. When the game is played only once or a determinate number of times, defection (ALLD) is the only ESS. On the other hand, if there is some probability of future interactions the game is transformed into a new meta-game known as the Iterated Prisoner's Dilemma (IPD) consisting of repeated bouts of the PD. In the IPD, ALLD is no longer the only effective strategy to play (Axelrod, 1980a; Axelrod, 1980b).

The most commonly discussed strategy for the IPD is tit-for tat (TFT) (Axelrod, 1980a; Axelrod, 1980b; Axelrod and Hamilton, 1981). TFT is an example of reciprocal altruism where individuals take turns performing altruistic acts towards each other (Trivers, 1971). TFT is a good strategy to play in the IPD for several reasons. It is a cooperative strategy allowing it to obtain the cooperator's payoff in a population of cooperators. It cooperates on the first move and cooperates if the opponent cooperates on subsequent moves. The cooperative nature of the TFT strategy allows it to obtain the Cooperate/Cooperate payoff in a population of TFT players or other cooperative strategies. Just as importantly, it is retaliatory strategy. If the opponent defects (cheats), TFT immediately retaliates by defecting in the next round. This aspect of the TFT strategy limits the damage a defecting strategy can inflict and allows a population of TFT to resist invasion by uncooperative strategies. This is because an uncooperative strategy would achieve higher fitness in any single interaction with TFT, TFT maintains higher overall fitness if the frequency of individuals playing TFT in a population is above some critical threshold. Additionally, the more likely individuals are to interact in the future the less impact the initial defection has, further enhancing the stability of TFT. Finally, TFT is a forgiving behavior. If the opponent cooperates after it has defected, TFT will cooperate in the following round (Axelrod and Hamilton, 1981). This provides some protection against mistakes if the opponent continues to cooperate.

In order to determine if an apparently altruistic behavior is a case of reciprocal altruism, it is necessary to show retaliation in response defection (Connor, 1986; Rothstein and Pierotti, 1988). Simply demonstrating reciprocity is insufficient because many examples of by-product mutualism appear to be reciprocal in nature (Connor, 1986). By-product mutualism is a special case of mutualism where a behavior appears to be altruistic on the surface, but on closer inspection the behavior actually benefits the "altruist" immediately and only benefits the recipient as a side effect. By-product mutualism frequently appears reciprocal as well, in which case it is referred to as pseudo-reciprocity (Connor, 1986). Lack of retaliation in response to defections would suggest that the behavior is in fact a form of mutualism and there is no temptation to cheat (Connor, 1986; Connor, 1995; Rothstein and Pierotti, 1988).

Many studies have shown retaliation in response to induced or simulated defections (reviewed in Dugatkin, 1997). For instance, predator inspection in guppies, *Poecilia reticulata*, (Dugatkin, 1991; Dugatkin and Alfieri, 1991a; Dugatkin and Alfieri, 1991b) and sticklebacks, *Gasterosteus aculeatus,* (Milinski, 1987), food sharing in vampire bats, (Wilkinson, 1984) and reduced aggression among territorial hooded warblers, *Dendroicha petechia*, (Godard, 1993). Godard (1993) showed that hooded warblers, *Dendroica citrina,* use TFT like strategies in territorial relationships. Male hooded warblers increased their aggressiveness towards playbacks of a neighbor's song at their common boundary after simulated intrusions by that neighbor, but not after simulated intrusions by a strange male. To date this is the only experimental demonstration of TFT like strategies being used in reduced aggression among "dear enemies".

Most studies of “dear enemy” relationships emphasize that neighbors respect each other’s borders by not expanding their own territory. While this is likely true, neighbors may also respect one another’s boundaries by refraining from seeking extra-pair copulations (EPCs) with their neighbor’s mates. Many studies in recent years have shown that extra-pair paternity is common in many species of birds (summarized in Moller, 2000). The cryptic nature of cuckoldry could allow a higher level of cheating within a stable system of cooperation. TFT and similar strategies are generally considered “honest” strategies but little attention has been paid to the effect that an inability to detect defectors might have on cooperation.

In this study, we determined if red-winged blackbirds (*Agelaius phoeniceus*) exhibit TFT-like behavior by reducing their aggression toward their neighbors. We used simulated invasions by neighbor and stranger males to test for levels of retaliation in response to defections in “dear enemy” relationships. In addition, determined whether males alter their behavior towards their neighbor in response to simulated invasions the day following the trial by observing natural territorial behavior before and after experimental manipulations. We also looked for evidence of cheating by determining the overall rate of extra-pair paternity in the population using analysis of microsatellite loci to establish paternity of nestlings. Red-winged blackbirds are known to have high level of extra-pair paternity (Gray, 1997; Weatherhead et al., 1994). Finally, we tested the hypothesis that “dear enemy” relationships are influenced by extra-pair paternity. If males are able to detect extra-pair paternity, then they should increase their level of aggression toward cuckolding males.

METHODS

Study Site and Population

This study was conducted at the Kellogg Biological Station Experimental Pond Facility, Hickory Corners, Michigan (42° 24' N, 85° 24' W) from 1998 and 1999. Each of 18 ponds was approximately 30 m in diameter and 3 m deep. The ponds were arranged in three rows of six. Within rows, ponds were approximately 5 meters apart and rows were spaced approximately 10 meters apart. The margin of each pond was densely vegetated, predominately with cattails (*Typha latifolia*).

Although the ponds were constructed primarily for aquatic research, they were consistently colonized by red-winged blackbirds. During this study, one to four males settled each pond and one to six females settled on each territory. A chain link fence surrounded the facility keeping out most predators, therefore nesting success was very high (near 100%).

Capture of birds, marking and blood collection

We began capturing males as they arrived using both walk-in traps baited with corn and with mist nets. Bait piles for the walk-in traps consisted of cracked corn and were placed so that each pile was equidistant from four ponds, except on the edges of the array where they were placed equidistant from two ponds. Males and females from all adjacent ponds readily visited the bait piles.

Upon capture, we marked it each bird with a unique combination of three colored leg bands and a numbered aluminum band. Age and sex were determined using plumage criteria (Pyle, 1997). In 1998, we drew 50-80 µl of blood from the brachial vein of all territorial males and immediately stored the sample in 800 µl of "Queen's" lysis buffer (Seutin et al., 1991). We drew approximately 0.5 µl of blood from nestlings when they were 8 days old and immediately placed samples in lysis buffer.

We mapped each male's territory using behavioral criteria. The entire pond was defined as a male's territory when he was the only resident male on that pond. On ponds with more than one male, we defined territorial boundaries where two neighboring males counter-sang (both males within 10 m of each other and singing in alternate order), at the limit of the resident's movement or at the center of the overlap of activity between two neighboring males.

We searched for nests daily after the arrival of females. We located most nests as they during construction and all nests were located within a week of completion. Therefore, we are reasonably certain that we found all nests. We marked the location of each nest with flagging tape at the edge of the pond and we monitored nests every 4 to 8 days until it's fate was determined. The majority of first brood nests were started on or near May 1. Although females frequently attempt a second clutch, we incorporated data from only the first brood to limit pseudo-replication and differences among broods.

Observation of territorial behavior

We observed male territorial behavior in 1998 and 1999. In 1998, we observed each male twice in random order from a blind approximately 10 m from the male's territory. All observations were made between 6 and 10 am during the first week of May when egg laying was at its peak. In 1999, we observed all territorial males for 20 minutes (twice for 10 minutes) in late April, just prior to the experiments described below. This is slightly before the period when males were most susceptible to being cuckolded (Gray, 1997) but allowed us to complete pre-trial observations so that we could perform experiments when egg laying was at it's peak. We also performed and additional 20 minute observation the day following the experiment.

An observational period consisted of a 3-minute acclimation period followed by the observational period. We recorded the position of the male, time spent at that position, the number of songs, song displays, counter-songs, fights and chases at that position and whether the male was foraging or vigilant. We also recorded the identity of the other male for counter-songs, fights and chases. In the few instances where the male was absent for an observation, we returned the next day to observe that male.

Paternity analyses

We determined paternity of nestlings using six microsatellite loci. Four of the loci (Qm 5, Qm 10, Qm 21, Qm 31) were developed for great-tailed grackles (*Quiscalus mexicanus*) (Gibbs et al., 1997), Dpμ 16 for yellow warblers (*Dendroica petechia*) (Dawson et al., 1997) and Maμ 10 for brown-headed cowbirds (*Molothrus ater*) (Gibbs et al., 1997).

DNA was extracted from the blood samples using Proteinase K digestion followed by extraction in 7.5M NH_4A_0C and precipitation in isopropyl alcohol. The DNA was washed once more in 70% ethyl alcohol.

We assayed genetic variation at these loci using PCR amplification in 25 μL reaction volumes. Two different reaction conditions were required for the six loci. Qm 10, Dpμ 16 and Maμ 10 were amplified using 250 ng of template DNA, 2 pmol of each primer (fluorescently labeled forward primer), 500 μM dNTPs, and .75 U Taq polymerase. Qm 5, Qm 21 and Qm 31 were amplified using 125 ng template DNA, 1.25 pmol of each primer (fluorescently labeled forward primer), 625 μM dNTPs and .75 U Taq polymerase. All reactions were performed in buffer containing 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 30mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01 Triton X-100. The PCR product was run on 6% polyacrylamide gels and visualized using an FMBIO gel scanner. We assigned genotypes to all individuals based on size comparisons with internal standards and individuals of known genotype run on the same gel. All gels were scored by hand by two authors (RO & KS) and verified using FMBIO image analysis software.

We included only territorial males as potential fathers, since previous work has shown that floating males rarely gain fertilizations (Gibbs et al., 1990). We were able to establish paternity for all but two of 252 offspring for which we obtained DNA. For 217 of the young, there was only one non-excluded candidate parent. We determined the paternity of the remainder of the offspring employing maximum likelihood methods using Cervus (Marshall et al., 1998). We performed maximum likelihood estimates of the remaining offspring using error rates of 0% and 0.5% resulting in probabilities of false inclusion (assigning the wrong father to the nestling) of 0.7% and 10.5% respectively. We were able to assign paternity to most of the remaining offspring with 95% confidence level

(26 offspring), but we also included assignments at the relaxed confidence of 80% (7 offspring).

Recording and editing of songs

We recorded the territorial songs of all territorial males in early April using a Sony Audio Acoustica 815a shotgun microphone and a Marantz PMD222 tape recorder. All recordings were made from a blind between 6 am and 10 am on calm days.

We uploaded several examples of each male's territorial song into .wav files at 16 bit resolution. We then filtered the samples to eliminate background noise with minimal distortion of the signal and standardized all songs to equal maximum amplitude using Cool Edit© sound editing software. We constructed playback files for each male by splicing 10 seconds of silence between each example of a male's song (minimum of 4 examples/male) resulting in a song rate of 6 songs/minute. This is within our observed range of male singing rate.

Simulated Defection Trials

We performed simulated territorial intrusions using a modified version of the playback experiment performed by Godard (1993; Figure 1) to test whether males retaliated in response to defection by a neighbor. We conducted all trials during the first week of May after we had observed every male for 20 minutes. We conducted all trials between 6 and 10 am and only used males that had neighbors on the same pond. We performed all trials from a blind approximately 10 m from the focal male's territory using a laptop computer running Cool Edit© software and Panasonic battery powered computer speakers to generate the stimulus. We set the volume by ear so that it was similar in volume to natural male songs and volume was kept constant throughout the experiment.

Prior to running trials, we began catching males using walk-in traps. Upon capture of a suitable male designated as the neighbor male and chose another male on the same pond as focal male. In order to avoid pseudo-replication we used each male only once as a focal male and only once as a neighbor male. We then assigned the pair to experimental and control groups (explained below) in alternating order. The neighbor male held in a covered cage in a building nearby so that it would not interact with the focal male during the experiment.

Each trial consisted of three playbacks referred to as pre-invasion, invasion and post-invasion playbacks (Figure 1). We waited 15 minutes after setting up the apparatus to perform the first playback and we waited 45 minutes between each successive playback. Each playback lasted 3 minutes.

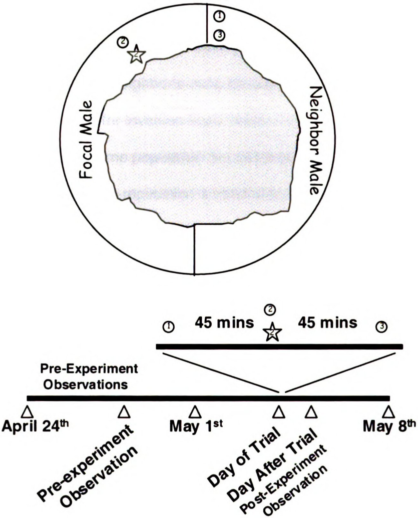

Figure 1. Schematic and time line of the experiment. Two territories are shown. Pre-experiment observations were performed between April 24 and April 30. All trials were conducted between May 1 and May 8. Numbers in the pond schematic indicate the order playbacks. Number 1 is the pre-invasion trial, number two is the invasion trial and number 3 is the post-invasion trial. Circles indicate that the neighbor's song was played at that position, the star indicates that a stranger's song was played. The time line shows an example of the timing of the observations and playbacks for one trial.

We placed the speaker on the neighbor's side of the territorial boundary for pre- and post-invasion playbacks while in invasion trials we placed the speaker approximately 10 m within the focal male's territory. In experimental treatments, we played the neighbor's song in all three playbacks (NNN). For control treatments, we used the neighbor's song for pre and post invasion trials, but used a stranger's song for invasion trials (NSN). The stranger's song was a male's song from the same population but not adjacent to the focal male's territory. To avoid pseudo-replication a male was used only once as a stranger's song (Kroodsma, 1989; Kroodsma et al., 2001). By using a song from the same population for a control, we ensured that the song could potentially be perceived as a viable threat to the focal male's territory.

For each trial we recorded latency of response to the playback (defined as an approach of 10 m or half the distance to the speaker) and closest approach to the speaker. We also recorded the number of songs, displays, hovers over the speaker, and any calls given by the male. Upon completion of the trial, we released the captured male and verified that it had returned to its territory by the afternoon following the experiment.

The day following an experiment, we observed the focal male's territorial behavior for 20 minutes as outlined above. We therefore have five points in time recorded for each replicate: a pre-trial observation, response to pre-invasion, invasion and post invasion playbacks, and a post-trial observation. In once case, the neighbor male was not present during the post-trial observation so we eliminated this trial from the analyses.

Statistical Analysis

We used correspondence analysis (CA) to reduce the number of variables analyzed. CA is similar to principle component analysis (PCA) in that it finds orthogonal axes (latent variables) that best explain the variation in multi-dimensional data. Correspondence analysis, however, may be more appropriate than PCA in situations where the component variables are not linearly related (Terbraak, 1985). For instance, aggressive behavior towards neighbors can be measured using several component variables. Some behaviors may only be exhibited at low levels of aggression (such as songs or displays) while others may only be exhibited at higher levels of aggression (such as physical attacks). This situation would violate the assumptions of PCA but would be well suited to analysis using CA. Additionally CA will often do a better job of explaining the variance in these situations.

To analyze the observational data we first determined each male's territorial behavior towards each of its neighbors from the behavioral observations. We determined the amount of time a male spent within 5 m of a neighbor's territory, how many songs and displays it performed in that area and how many counter-songs, chases and fights it had with that neighbor. We then performed separate correspondence analyses for each year of observational data, 1998 and 1999. Behavioral observations within a year were analyzed together to facilitate comparisons.

We analyzed both the first correspondent dimension (CD1) and the second correspondent dimension (CD2) (Table I). CD1 accounted for 45-50% of the variation in the data and the component variables always loaded such that CD1 was associated with increased aggression. We therefore refer to CD1 as aggression. CD2 did not yield consistent results between years for the observational data. Additionally, CD2 never yielded significant results in any of the statistical tests for observational or experimental data. We therefore do not refer to it further.

To test for treatment effects in the experiment we used repeated measures analysis of variance (repeated measures ANOVA). Each playback or observation was considered a repeated measure of experimental or control treatments. We used pooled variance t-tests to test for significant differences within playbacks or observations when the repeated measures ANOVA yielded significant differences.

Since each male had several neighbors, there were multiple pair-wise observations for each male. We used nested ANOVA to test for differences in aggression between males towards neighbors who had cuckolded them and

Table I. Component loadings and percent of variance explained by the correspondence analyses performed on observational data in 1998 and 1999 (**a**) and experimental data (**b**).

	CD1	CD2	CD1	CD2
Time Spent at Border	0.18	0.03	0.29	0.77
Time Spent Foraging	-0.21	0.20	-0.56	-0.65
Time Spent Off Territory	-0.17	0.04	-0.24	-0.05
Songs at Border	0.28	0.02	0.81	-0.07
Counter Songs	0.16	0.06	0.48	-0.05
Displays	0.28	0.04	0.57	-0.08
% Variation Explained	44.49	22.76	48.13	25.35

b.

Experimental Data

1999.00

	CD1	CD2
Latency of Response	-0.56	-0.43
Closest Approach	-0.55	-0.34
Songs	0.05	0.21
Hovers	0.72	-0.38
Displays	0.51	0.68
Calls	0.68	-0.48
% Variation Explained	50.85	28.40

males that had not. Levels of aggression between cuckolding and non-cuckolding neighbors were nested within each male. We used analysis of covariance to test for a relationship between aggression by a male towards a neighbor and the neighbor's ability to gain extra-pair fertilizations (EPFs) across the entire population and aggression. In this case, each male was used as a categorical variable and number of EPFs obtained by the neighbor was the covariate. All ANOVAS were run as random effects models in SAS.

RESULTS

Males responded more aggressively to playbacks at the territorial boundary 45 minutes after simulated intrusion by a neighbor than after simulated intrusion by a stranger (repeated measures ANOVA: Treatment $F_{1,11} = 12.914$, $P < 0.005$; Trial $F_{2,22} = 0.066$, N.S.; Treatment * Trial $F_{2,22} = 15.103$, $P < 0.001$; Figure 2). Males from both treatment groups behaved identically in the pre-invasion trial (t-test: $t_{11}=1.036$, N.S.). Males behaved more aggressively in both the invasion trial (t-test: $t_{11} = 4.276$, $P < 0.001$) and in the post-invasion trial (t-test: $t_{11} = 6.084$, $P < 0.001$). Although overall aggression differed significantly among treatments, the component behaviors did not differ significantly (t-test: t_{10}, N.S. in all cases). For each variable, however, the difference between treatments was in the predicted direction, the cumulative effect of which leads to the significant differences observed when analyzing aggression. Males therefore increased aggressive behavior against playbacks of their neighbor's song after

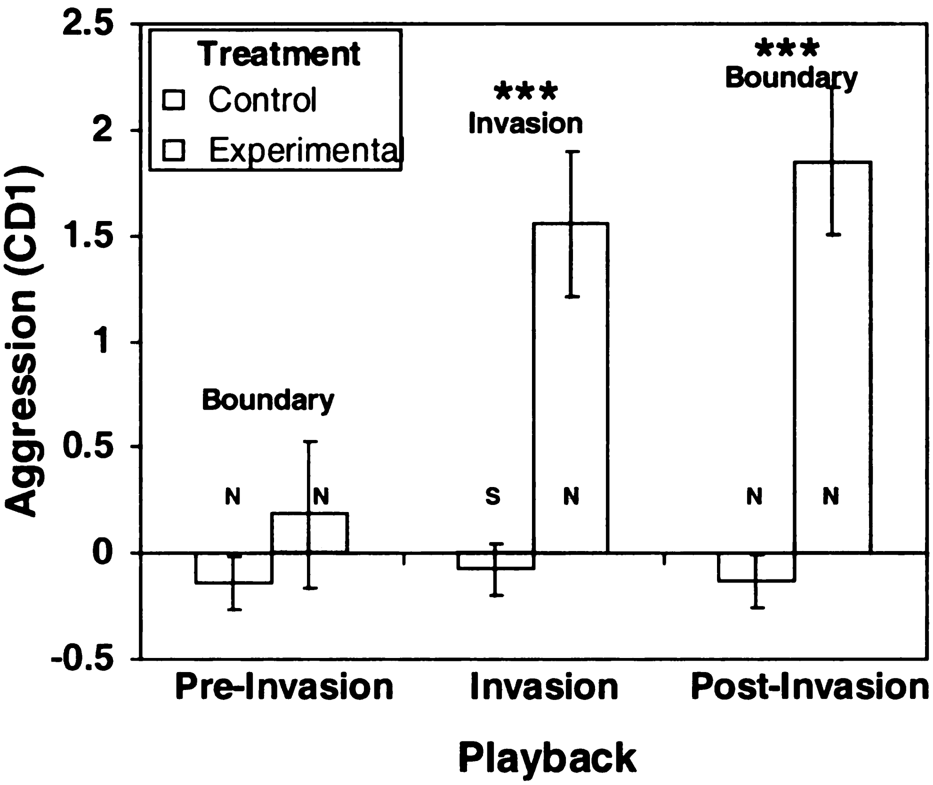

Figure 2. Aggression by focal males toward playbacks in response to experimental treatment. Experimental treatments simulated territorial invasions by neighbors, control treatments simulated invasion by stranger males. Position of the speakers is given above each pair of bars. The song played in a playback is shown inside or above each bar, N = Neighbor, S = Stranger. Error bars give standard error. N = 6 in both treatments. *** P < 0.001.

simulated invasion by the neighbor, but did not exhibit increased aggression to the playbacks of their neighbor's song after simulated invasions by strangers. Additionally, focal males did not react aggressively against stranger playbacks during the invasion trials but reacted very aggressively against neighbor playbacks in invasion trials.

The results above show that males react more aggressively to playbacks of their neighbor after defection, but these results do not show that males alter their behavior towards their actual neighbor. We therefore used observations of territorial behavior between the focal and neighbor male before and after simulated defections. The level of aggression toward neighbor males increased significantly for both treatments, however the increase was greater for males in the experimental group (repeated measures ANOVA: Treatment $F_{1,10} = 2.147$, N.S.; Trial $F_{1,10}= 15.551$, $P < 0.001$; Treatment * Trial $F_{1,10}= 17.425$, $P < 0.005$; Figure 3). Except for display rate, which differs slightly in the other direction, the component behaviors differ in the expected direction but not significantly (t-test$_{11,}$ N.S. in all cases). Males therefore not only respond more aggressively towards playbacks of their neighbor's song in response to defection by their neighbor, but also behave more aggressively towards their neighbor the day following the experiment.

We collected blood from 252 offspring, 36.2% of which were products of extra-pair fertilizations. Of 93 nests, 52.4% contained cuckolded young. To determine

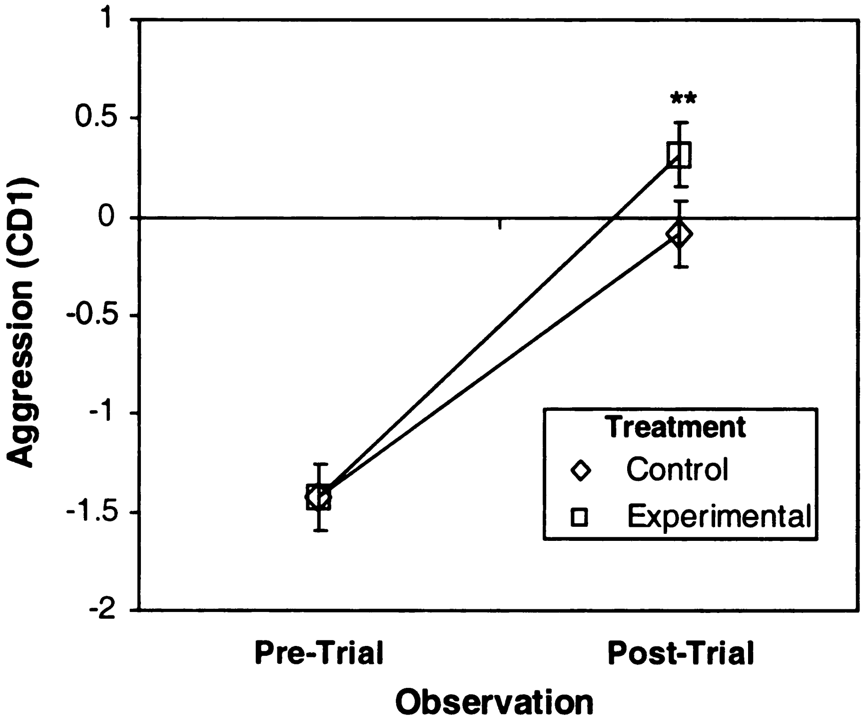

Figure 3. Aggression by the focal male directed towards its neighbor before and after simulated invasions in response to treatment. Males in experimental treatments had songs from a neighbor played within its boundaries. Males in control treatments had songs from a strange male played within its boundaries. Error bars give standard error. N = 6 for both treatments. ** $P < 0.01$

if the level of aggression between males was correlated with cuckoldry, we analyzed interactions between neighboring males on the same pond or closely adjacent ponds. To control for distance effects of both aggression and cuckoldry, we did not include neighbors from different rows. The territorial behavior between males and the paternity analysis were performed separately and effectively blind of each other. The results show that males are more aggressive towards neighbors that have successfully cuckolded them (nested ANOVA: male $F_{18,19}$= 1.24, N.S.; cuckoldry(male) $F_{19,42}$= 2.32, $P < 0.05$; Figure 4).

We reasoned that total EPFs obtained by a male across the entire population would correlate with a male's attractiveness to females. We therefore tested to see if there was a correlation between aggression towards a neighbor and the total number of EPFs that neighbor obtained. There was a positive correlation between aggression towards a neighbor and that neighbor's ability to gain EPFs (ANCOVA; male $F_{19, 59} = 0.826$, N.S.; neighbor's EPFs $F_{1,59} = 4.465$, $P < 0.05$; male * neighbor's EPFs $F_{19, 59} = 0.828$, N.S.; Figure 5).

DISCUSSION

The results of our study parallel those of Godard's (1993) study. Red-winged blackbirds, like hooded warblers, use TFT-like strategies in reducing their aggression toward their neighbors. In addition, our study extends Godard's

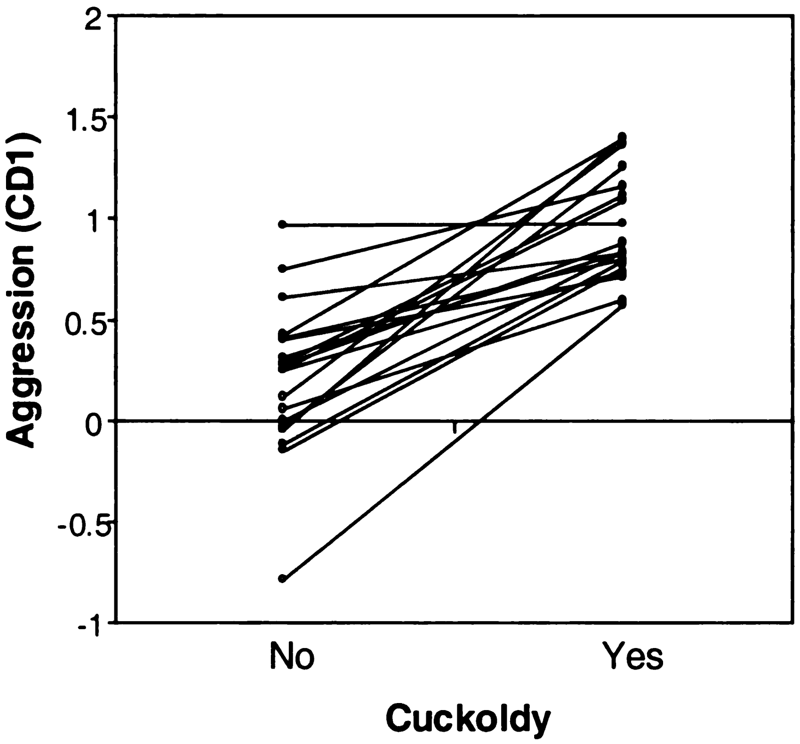

Figure 4. Average aggression by each male toward neighbors who sired young on its territory and those that had not. Each line represents one male and each circle represents the average aggression towards neighbors in that category. Three males are not shown because one was cuckolded by all of its neighbors and two males were not cuckolded by any of their neighbors. N = 19 males.

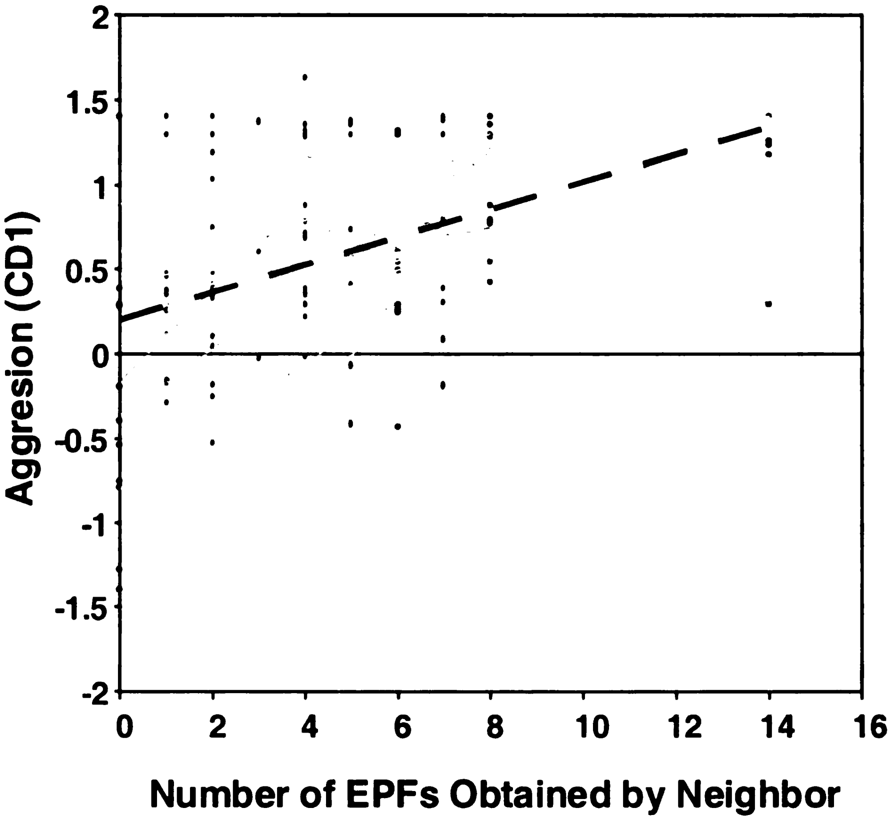

Figure 5. Aggression by territorial males in response the total number of extra-pair fertilizations obtained by a neighbor across the entire population. The circles show all pair wise interactions (N=99). The dark dashed line shows the over all trend the solid gray lines give show the trend lines for each individual male (N= 22).

work in several ways. Although Godard mentions that hooded warblers seemed to act more aggressively towards their neighbors after simulated intrusions, this was not tested explicitly. We show that the response to defection is not just directed towards the experimental playbacks, but also towards the neighboring male the day following the experiment. We also evaluated effect of cheating on “dear enemy” relationships. Our study suggests that males are sensitive to cheating by their neighbors and increase their aggression towards cuckolding neighbors. This response may, however, reflect the capability of males to accurately determine the ability of its neighbors to obtain EPFs rather than a male’s ability to detect cuckoldry directly. Males appeared to increase their defense towards “sexy” neighbors that were able to gain large numbers of EPFs overall and would presumably be better able than “less sexy” neighbors to cuckold his offspring.

Do red-winged blackbirds play TFT like strategies?

The three crucial traits that make TFT such an effective strategy to play in the IPD are; 1) it cooperates with cooperative strategies 2) it retaliates in response to defection and 3) forgives when the neighbor again cooperates (Axelrod and Hamilton, 1981). This study suggests that red-winged blackbirds have at least 2 of these three attributes, they are initially cooperative and retaliate in response to defection. They do not, however, appear especially quick to forgive.

Males were generally cooperative in that their levels of aggression towards their neighbors were low compared to their aggression towards males that have defected. In both pre-trial observations and in pre-defection trials, focal red-winged blackbirds showed little aggression towards one another or to the playback. Most of their time was spent singing from a perch well within their territory, foraging or chasing females. In contrast, males increased their aggression markedly in response to the invading song from a neighboring male, while not reacting at all to the song of an invading stranger.

Males retaliate against cheaters following territorial invasions. In response to simulated invasions by their neighbors, male red-winged blackbirds significantly increased their level of aggression towards that neighbor in post-invasion trials and continued to behave more aggressively the following day. This increase was pronounced, focal males approached the speaker very closely, often perching on top of it and appeared to search the area closely for the offending male. This result is consistent with Godard's (1993) results.

Although we did not specifically study forgiveness, red-winged blackbird males do not appear to quickly forgive an invasion by their neighbor. In this study, where we simulated invasion by neighboring males, focal males continued to behave more aggressively towards their neighbors on the day following the experiment compared to males where we simulated invasions by stranger males. Although not explicitly testing this either, Godard (1993) noted that males also appeared to show increased aggression towards neighbors following simulated invasions. The concept of forgiveness, however, is not well developed. Clearly returning to background levels of aggression would be interpreted as forgiveness, but there is no a priori way of knowing how long increased levels of aggression should last.

Perhaps the best interpretation of our result is that after the apparent defection by a neighbor, the pair must start over and negotiate reduced aggression anew. This process may take a few days through incremental investment in cooperation (Roberts and Sherratt, 1998). A better framework to understand forgiveness in continuous games (?) may be to model forgiveness using negotiation rules as opposed to action rules. Negotiation rules can model the rate at which aggression decreases as well as the final level of reduced aggression. Action rules, on the other hand, model the response to defections (McNamara et al., 1999).

Cheating among "Dear Enemies"

Implicit in TFT is a low rate of cheating (defecting), because cheating should be selected against in a TFT (or TFT like) population. Within any single interaction, a cheater will always achieve higher fitness than an individual playing TFT. However, TFT individuals enjoy a higher long-term payoff in the IPD, because every time two TFT strategists meet they receive the cooperator's payoff. If the frequency of TFT individuals is high enough, TFT will be favored over uncooperative strategies (Axelrod and Hamilton, 1981). This of course assumes that cheating is detected. If cheating is difficult to detect, as is the case with cuckoldry, cheaters may go undetected. However, if males invade their neighbor's territory to expand their own territory, cheating should easily be detected.

The resource being guarded in our study or the motivation behind any territorial invasions that might occur is unknown. Godard (1993) and Getty(1987) assume that males are guarding their physical territory. However, their reasoning does not exclude the possibility that they may be guarding access to their females as well.

Our study shows that males behave more aggressively towards neighbors that have cuckolded them compared to males that had not. This is a surprising result given that extra-pair copulations (EPCs) are cryptic events. Our study and Gibbs' (1990) show that most EPFs come from neighbors. In addition, Gray (1996) observed that 78% of EPCs occur off the territory and that females appear to actively solicit them and control the level of extra-pair paternity in their young. These results suggest that males may be unable to directly determine which males have cuckolded them. Males may, however, be able to assess which neighbors are most attractive to females and behave more aggressively towards those "sexy" males.

How males are able to assess on another is uncertain. Larger and older males tend to sire more total young (Weatherhead and Boag, 1995), however it is unknown if males are responsive to size. The evidence for physical characters contributing to territory acquisition is weak (Eckert and Weatherhead, 1987a; Eckert and Weatherhead, 1987b) and may therefore not factor into a male's assessment of a neighbors ability to gain EPCs. Alternatively, males may assess other male's behavior to gauge their resource holding potential (Freeman, 1987) through behavioral interactions with other males and may use similar strategies and monitor their neighbors' behavior to assess a neighbors' ability to achieve EPCs.

Despite the fact that red-winged blackbirds do not appear to be particularly honest in one aspect of their "dear enemy" relationships, they continue to maintain relatively low levels of aggression with each other. Perhaps the benefits of reduced aggression are great enough that males will tolerate a certain level of cuckoldry. In environments were there was a strong advantage to cooperating, guppies were more tolerant of defection (more forgiving) compared to environments were cooperation was less beneficial (Dugatkin and Alfieri, 1992). Additionally, since females appear to seek EPCs away from the territory, there may be little males can do about it except to seek their own EPCs.

Neighbor recognition and “Dear Enemies”

Several studies have shown that territory owners react more strongly to strangers on their border than to neighbors (Brindley, 1991; Fox and Baird, 1992; Godard, 1991; Stoddard et al., 1991). By reducing aggression towards their neighbors, males can reduce the cost of territorial defense. On the other hand, strange males on the boundary may be new males attempting to establish a territory that could intrude onto the resident male’s territory and are therefore present more of a risk.

One striking result of our study is the focal male’s lack of interest in strangers present their territory. Although our study never determined the behavior of males towards strangers on their territorial boundaries, hooded warblers react strongly to strangers on the border (Godard, 1991) but not to strange males are invading (Godard, 1993).It is possible that strange males within the territory are not perceived as a threat. In fact, in our study non-territorial males were frequently observed on territories and were seldom harassed by the resident male. We also never observed new territories being added after initial establishment in our population. Perhaps stranger males in the territory are not perceived as a threat because they are unable to usurp an entire territory and are unlikely to gain EPCs from the resident females.

This is the first study to show that the increased aggression displayed to playbacks in simulated invasions is also transferred to the neighboring male that appeared to invade. While males respond strongly to the songs of neighbors on their territory, they do not react strongly to the songs of strange males on their territory. This may be a cost saving trait as stranger males may present little risk to territorial males. We found a relatively high level of cuckoldry in this population that we interpret to be cheating and there is a positive relationship between cuckoldry and levels of aggression. Males behave more aggressively to males that have succeeded in gaining EPCs on its territory than those that did not. However, males also behaved more aggressively towards males that were most successful in gaining EPCs overall suggesting that males do not detect cuckoldry directly but rather are able to asses a neighbors attractiveness to females and react more aggressively towards attractive males.

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CHAPTER 3:

COOPERATIVE NEST DEFENSE IN RED-WINGED BLACKBIRDS: SIMULTANEOUS TESTS FOR RECIPROCAL ALTRUISM AND MUTUALISM

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ABSTRACT

Male red-winged blackbirds frequently cooperate in defense against a nest predator. Previous studies suggest that such cooperation can result from either reciprocal altruism or mutualism. While theoretically distinct, the two mechanisms are not mutually exclusive and may act in concert to favor high levels of cooperation. We tested for both tit-for-tat like strategies (a form of reciprocal altruism) and by-product mutualism. We used simulated defections to test for reciprocal altruism and determined paternity based on microsatellite analysis to examine potential causal mechanisms underlying mutualistic interactions among males. Results show that male red-winged blackbirds cooperate in nest defense primarily as a form of reciprocal altruism. In trials

where we simulated defections by neighboring territorial males, males reduced their level of defense against a nest predator at a common territorial boundary the day following the simulated defection. In contrast, males where defense was not manipulated increased their level of defense over the same period. We found no evidence that male red-winged blackbirds defend nests where they have sired young or that they reduce defense at cuckolded nests on their own territory. Considered with other studies, the results of this study suggest that different forms of cooperation may evolve in different populations. This suggests that other studies that test for both reciprocal altruism and mutualism may be of considerable value.

INTRODUCTION

Frequently when a potential nest predator enters a breeding colony of birds, it is "mobbed" by more than one adult. By cooperating in nest defense, otherwise competitive neighbors may benefit from reduced nest predation. However, there may also be temptation to cheat, relying on others to drive off the nest predator and avoiding the costs associated with nest defense. Such simultaneously competitive and cooperative relationships between neighboring males are often referred to as "dear enemy" relationships (Fisher, 1954; Getty, 1987).

Red-winged blackbirds, *Agelaius phoeniceus*, are colonial and males often develop relationships both within and across breeding seasons (Beletsky, 1989). Long-term associations can favor the evolution of "dear enemy" relationships through reciprocal altruism (Getty, 1987; Trivers, 1971). Reciprocal altruism is a form of cooperation where an individual helps another when there is a reasonable expectation that the other individuals will reciprocate helpful act in the future. With repeated interactions, both individuals will achieve higher fitness than individuals that don not cooperate.

If cooperative nest defense is primarily a form of reciprocal altruism, then it can by modeled using the iterated prisoner's dilemma (IPD). The IPD is an extension of the prisoner's dilemma (PD). The PD was first used to model economic and social behavior (Von Neuman and Morgenstein, 1953) and later applied to evolutionary theory (Axelrod and Hamilton, 1981; Maynard Smith, 1982). Trivers (1971) makes use of the concept in developing the theory of reciprocal altruism. In the PD and IPD, if both individuals cooperate, they both receive higher fitness (R) than when both defect (P). If one individual defects while the other cooperates, the defector receives the highest possible payoff (T) while the cooperative individual receives the lowest payoff (S). The resulting scenario conforms to the inequality ($T>R>P>S$). An additional constraint ($2R > T+S$) is often included to insure that two individuals cannot do better than always cooperating by alternating defecting and cooperating behaviors. Pure cooperation is not evolutionary stable in the PD, but in the IPD, several stable cooperative strategies have been found (Axelrod and Dion, 1988; Axelrod and Hamilton, 1981).

The most commonly discussed of which is the tit-for-tat strategy (TFT), popularized by Axelrod (1980a; 1980b) and further developed in an evolutionary context by Axelrod and Hamilton (1981) and as a potential evolutionary stable strategy (Maynard Smith, 1982). TFT is a strategy where an individual cooperates on the first move, and copies it's opponent's last move thereafter. TFT is a good strategy in the IPD. In a population of individuals engaging in TFT-like behavior, or other cooperative strategies an individual always cooperates, yielding a high payoff. In the presence of a defecting strategy, however, an individual limits the damage by only cooperating on the first move and defecting thereafter.

Several studies show that birds are capable of recognizing their neighbors (Brindley, 1991; Godard, 1991; Stoddard et al., 1991). Beletsky and Orians (1989) show that males with familiar neighbors enjoy higher nesting success than males with unfamiliar neighbors suggesting that familiarity might favor cooperation among neighbors. These studies suggest that red-winged blackbirds might use TFT-like strategies in cooperative nest defense. Weatherhead (1995), however, found the reverse pattern, whereby males with at least one unfamiliar neighbor had higher nest success due to reduced nest predation.

An alternative to reciprocal altruism described above that may be consistent with the Weatherhead (1995) study is pseudo-reciprocity. Pseudo-reciprocity is a form of mutualism where individuals appear to take turns helping one another, while in fact, each individual benefits directly from helping (Connor, 1986; Connor, 1995). Weatherhead (1994)showed that nests that containing young from extra-pair matings benefited from higher nest success than nests that contained only young from the resident male. Weatherhead suggested that males defend nests on other territories when they have sired young in them. Gray (1997) showed that males are more likely to help defend nests where they have sired extra-pair offspring. These helpers would appear to be incurring a cost by helping at another male's nests while in fact they are defending their own young from nest predators. Any benefits that accrue to the recipient would be a by-product of the helper's behavior.

Pseudo-reciprocity and TFT both appear reciprocal in that individuals help their neighbors and are in turn helped by their neighbors. Simply demonstrating reciprocity, therefore, does not distinguish between reciprocal altruism and mutualism (Connor, 1995; Rothstein and Pierotti, 1988). To demonstrate that a behavior is an example of TFT it is therefore necessary to show retaliation in response to defection. Lack of retaliation would suggest that there is no temptation to defect, that the behavior is directly beneficial to the helper and would be consistent with mutualism. Demonstrating the benefits, however, would constitute stronger evidence for mutualism. There are now many studies that have shown retaliation in response to simulated or induced defections and there are also several studies that have demonstrated mutualism (see Dugatkin, 1997, for a complete review)

Although theoretically distinct, mutualism and TFT are not mutually exclusive. It is feasible that mutualism may support a certain level of cooperation, but higher levels of cooperation might be achieved through TFT-like relationships acting in concert with mutualistic interactions resulting in increased frequency of cooperative interactions. For example, a helper may defend a neighbor's nest if the helper had sired young in that nest. This of course would benefit both individuals directly. That same helper might also help at the nest regardless of the paternity of the young if the neighbor could be counted on to help in the future.

Although there are many studies that test for either TFT-like behavior or mutualism, none simultaneously test for both. Experiments that simulate or induce defections to test for retaliation look for reduced cooperation by the "duped" individual. If there is a mutualistic component to the interaction, however, some level of cooperation may remain undetected by simulated defections. In order to detect mutualistic interactions, it is therefore necessary to show that there is a benefit to cooperating regardless of the behavior of the other individual.

In this study, we looked for evidence of both TFT and mutualism in cooperative nest defense in red-winged blackbirds. To test for TFT-like strategies, we looked for evidence of retaliation in response to simulated defections. To test for mutualism, we look for evidence that males are more likely to aid in defense of nests on other territories in which they have sired young.

METHODS

Study Site and Population

This study was conducted at the Kellogg Biological Station Experimental Pond Facility, Hickory Corners, Michigan (42° 24' N, 85° 24' W).The site consisted of 18 ponds that were approximately 30 m in diameter and 3 m deep. The ponds were arranged in three rows of six. Within rows, ponds were approximately 5 meters apart, rows were spaced approximately 10 meters apart. The margin of each pond was densely vegetated predominately with cattail (*Typha latifolia).* Although constructed primarily for aquatic research, red-winged blackbirds readily colonized the ponds. During this study, one to four males settled each pond and one to six females settled on each territory. A chain link fence surrounded the facility keeping out most predators, nesting success was therefore nearly 100%.

Collection of Background Data

We captured birds using both walk-in traps baited with corn and with mist nets. Bait piles were placed so that each pile was equidistant from four ponds, except on the edges of the array where they were placed equidistant from two ponds. Males and females from all adjacent ponds readily visited the bait piles.

Upon capture, we marked each individual with a unique combination of three colored leg bands for visual identification in the field and with a numbered aluminum band. Age and sex was determined using plumage criteria (Pyle, 1997). In 1999 we obtained 50-80 µl of blood from the brachial vein of all territorial males and samples were immediately stored in 800 µl of "Queen's" lysis buffer (Seutin et al., 1991). We obtained approximately 50 ml of blood from nestlings when they were 8 days old and immediately stored the samples in lysis buffer.

We used behavior criteria to map each male's territory. The entire pond was defined as a male's territory when he was the only male there. On ponds with more than one male, we defined territorial boundaries using the following criteria. We estimated boundaries to be at locations where two neighboring males counter-sang (both males were within 10 m of each other and sang repeatedly in response to each other's songs), at locations marking the limit of the resident's movements or at locations approximating the center of overlap between two neighboring males' area of activity.

We searched for nests when females arrived. The majority of first brood nests were started on or near May 1. We located most nests as they were built, and all nests were located within a week of completion. We found no nests later in the nesting cycle so we are reasonably certain we found all nests. We marked the location of each nest with flagging tape on the edge of the pond. Nests were monitored every 4 to 8 days until the fate was determined. Egg and nestling stages lasted approximately 11 days each and the nesting season ended in early July when the second broods fledged.

Paternity analyses

We determined paternity of nestlings using six microsatellite loci. Four of the loci (Qm 5, Qm 10, Qm 21, Qm 31) were developed for great-tailed grackles (*Quiscalus mexicanus*) (Gibbs et al., 1997), Dpμ 16 for yellow warblers (*Dendroicha petechia*) (Dawson et al., 1997) and Maμ 10 for brown-headed cowbirds (*Molothrus ater*) (Gibbs et al., 1997).

DNA was extracted from the blood samples using Proteinase K digestion followed by NH_4A_0C extraction and precipitation in isopropyl alcohol. The DNA was washed once more in 70% ethyl alcohol. We assayed genetic variation at each of six loci using PCR amplification in 25 μL reaction volumes. Two different reaction conditions were required for the six loci. Qm 10, Dpμ 16 and Maμ 10 were amplified using 250 ng of template DNA, 2 pmol of each primer (fluorescently labeled forward primer), 500 μM dNTPs, and .75 U Taq polymerase. Qm 5, Qm 21 and Qm 31 were amplified using 125 ng template DNA, 1.25 pmol of each primer (fluorescently labeled forward primer), 625 μM dNTPs and .75 U Taq polymerase. All reactions were preformed in buffer containing 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 30mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01 Triton X-100. The PCR product was run on 6% polyacrylamide gels and visualized using an FMBIO gel scanner. Genotypes were assigned to all individuals based on size comparisons with internal lane standards and individuals of known genotype run concurrently on each gel. All gels were scored by hand by both authors and verified using FMBIO image analysis software.

We included only territorial males as potential fathers; previous work has shown that floating males rarely if ever gain fertilizations (Gibbs et al., 1990). We were able to establish paternity for all but two of 252 offspring for which we obtained DNA. For 217 young (87%) there was only one non-excluded candidate parent. The remainder of the offspring were assigned paternity based on maximum likelihood estimates of paternity using Cervus (Marshall et al., 1998). We were able to assign paternity to 26 of remaining the offspring with 95% confidence level, but we also included assignments at the relaxed confidence of 80% for seven offspring. The two offspring for which no male could be assigned paternity were mismatched at two loci or more for all candidate parents. Although we could still assign paternity based on maximum likelihood estimates, we felt it was more likely that an unknown male sired these offspring.

Recording of songs

We recorded the territorial songs of all territorial males in early April 1999 using an Audio Technica AT815a shotgun microphone and a Marantz PMD222 tape recorder. All recordings were made from a blind from 6 am to 10 am.

After recordings were made, we uploaded several examples of territorial songs from each male into .wav files at 16 bit resolution. We then filtered as much noise as was possible from the recordings without reducing the quality of the song and standardized all songs to equal amplitude using Cool Edit © sound editing software. We then spliced a minimum of four song examples from each male together, separating each example with 10 seconds of silence resulting in a call rate of approximately six songs/minute.

Observation of cooperative nest defense

In order to determine each male's willingness to defend nests on other territories, in 1998 we performed presentations of stuffed crows at nests 1 to 4 days after hatching. Red-winged blackbirds substantially increased their willingness to defend after hatching and we were therefore more likely to observe helping in nest defense at this time. To minimize affects of repeated exposure to the stuffed crow (Knight and Temple, 1986), presentations performed on the same day were separated in distance by at least one pond. All presentations were performed between 6 and 10 am, when males were most likely to be on their territories. If any of the neighbors were not present for a presentation, we returned the next day to perform a presentation.

For each trial, we positioned a stuffed crow, mounted on a pole perched posture, within 1 meter of the nest. The crow was initially covered with a cloth with string attached to it. We then observed any reaction to the covered crow for 3 minutes from a blind 10 m from the crow. In no case was there any reaction to the covered crow. After 3 minutes, the cloth was pulled off and we observed any reaction to the crow by all neighbors for 3 minutes.

For each male responding to the crow, we recorded that male's identity, latency to approach, closeness of approach, number of vocalizations of reach type, hovers, dives and strikes. Red-winged blackbirds are known to use as many as seven defense calls (Knight and Temple, 1988; Orians, 1961) but for analyses we kept a tally for only the most common calls, the "Teer", "Seet" and "Titi" (Knight and Temple, 1988, and pers. obs. RO). We combined all other calls together excluding the ubiquitous "Chit" call which red-winged blackbirds use constantly. The "Chit" call is given almost constantly and is likely a contact call between male and female (Yasukawa, 1989). We therefore reasoned it would contribute little information on nest defense.

Experimental tests for retaliation

In 1999, we used simulated defections by neighboring males to test for retaliation. In order to reduce the effects of paternity and placement of nests we performed all trials at the territorial boundaries between males on the same pond. Placing the crow at territorial boundaries had the additional benefit that both males should benefit equally from defense so that any changes in behavior were attributable to the experiment. All trials were performed in late May when a substantial number of the nests contained nestlings.

To establish the baseline level of cooperative defense for each pair of males we first performed a crow presentation at every territorial boundary in the population (pre-defection presentation). The crow was covered and males were allowed to acclimate for 3 minutes. We then uncovered the crow and observed the behavior of both neighbors for 3 minutes. For each male we recorded the latency of response, closest approach, hovers, dives, strikes, "Teer", "Seet" and "Titi" calls as well as summing up all other calls given excluding the "Chit" call.

After establishing initial levels of defense among neighbors, we performed simulated defections. Before performing experiments, we began capturing males. When we captured a suitable, it was designated as the neighbor male, we determined it's neighbor (the focal male) and assigned them to experimental or control groups in alternating order. Males were used only once as focal males and neighbor males although some males were used as both focal and neighbor males. Captured males assigned to control groups were taken to a nearby building and released. Captured males assigned to experimental groups were taken to the same building, kept in a covered cage and given generous supplies of cracked corn and water.

We waited 30 minutes to perform the defection presentation and in the case of pairs assigned to control groups verified that the neighboring male had returned to the territory. We placed the covered crow on the pair's territorial boundary and placed a set of speakers well within the neighbor male's territory. We waited an additional 15 minutes to allow the males to recover from the disturbance and performed the defection presentation.

In control trials, we played ambient sounds recorded near the pond facility but well away from any red-winged blackbirds. To avoid pseudo-replication, a different example of ambient sounds was used for each playback (Kroodsma et al., 2001). In experimental trials, we played the neighboring male's territorial song. The goal in experimental trials was to make it appear as though the neighboring male was present but refusing to participate in defense against the crow. We observed the response of the focal male for 3 minutes recording the same data we recorded during pre-defection presentations. After completion of this playback, the captured male was released and we verified that it had returned by the afternoon.

The day following a simulated defection presentation, we performed the post-defection presentation. This presentation was conducted the same as the pre-defection presentation. The covered, stuffed crow was placed at the territorial boundary. We retreated to a blind and waited 3 minutes. We then uncovered the crow and recorded both males' reaction to the crow for 3 minutes.

Statistical Analysis

Since our behavioral observations consisted of many measures of nest defense behavior for each individual, we used correspondence analysis (CA) to reduce the number of dimensions in our data. CA is similar to principal component analysis (PCA) in that it reduces the number of dimensions in a multivariate data set. CA however is more appropriate than PCA when the component variables are not linearly related (Terbraak, 1985). In the case of nest defense, CA is more appropriate because certain component behaviors may only be expressed at low levels of aggression, while others may only be expressed at higher levels of aggression. In addition, CA explains a greater proportion of the variance under these circumstances.

We performed separate correspondence analyses on data from presentations made at individual nests(where we tested for a male's willingness to defend nests in which they had extra-pair copulations) and data from presentations at territorial boundaries (where we tested for TFT-like relationships among neighboring males). The correspondence analysis on presentations at territorial boundaries included data from both the focal and the neighbor male for each pair of neighbors. In all cases, the component variables correlated with the first correspondent dimension (CD1) such that an increase in CD1 was associated with an increase in nest defense (Table I). For clarity, we therefore refer to CD1 as defense. The second correspondent (CD2) dimension never correlated consistently with the component behaviors between data sets nor was it easily interpretable (Table 1). Nonetheless, we subjected CD2 to identical statistical tests as CD1, but CD2 never yielded significant results. We therefore do not refer to CD2 further.

Since each focal male in the defection experiment was used in pre-defection, defection and post defection presentations, we used repeated measures analysis of variance (ANOVA) to analyze the results. Presentations were blocked within male and we tested for effects of treatment, presentation and the interaction between the two. Likewise, we analyzed the affect of the experimental treatment on the neighbor male's behavior using repeated measures ANOVA.

To determine if neighboring males were more likely to defend an off territory nest in which they had sired offspring we used a paired t-test. For each nest or territory with extra-pair offspring, we paired the genetic father of cuckolded

Table I. Component loadings and percent of variance explained by the correspondence analyses performed on data taken from crow presentation at a) individual nests and b) territorial boundaries

a. Presentations at Nests

	CA1	CA2
Approach	-0.900	0.028
Latency	-0.873	0.061
Teer	0.629	-0.419
Titi	0.351	0.233
Seet	0.302	0.787
Hover	0.639	0.110
Dives	0.478	0.100
Strikes	0.519	-0.900
% Variance	43.05	17.55

b. Presentations at Boundaries

	CA1	CA2
Latency	-0.744	0.034
Approach	-0.531	0.296
Teer	0.518	-0.458
TiTi	0.516	0.803
Seet	0.352	-0.200
Hover	0.324	0.277
Dive	0.438	-0.365
Strike	0.240	-0.388
% Variance	37.80	28.65

offspring with a randomly chosen neighbor using a pseudo-random number generator. The neighbor's territory was the same distance from the nest and had not cuckolded that nest.

Because each male's territory consisted of several nests, the resident male might influence nest defense at nests within a territory. Therefore, for tests involving multiple nests within a territory we used nested ANOVAs to partition variance between nests within a territory and variation at the level of territory.

RESULTS

The results of the defection experiment revealed that males reduce their level of defense at their territorial boundary in response to their neighbor's lack of defense (repeated measures ANOVA, treatment $F_{1,11} = 2.571$, N.S., trial $F_{2,22} = 0.429$, N.S., treatment * trial $F_{2,22} = 4.132$, $P < 0.05$; Figure 1). Defense by the focal male was almost identical in the pre-defection presentation of the crow. Males in the experimental group significantly decreased their level of nest defense in the defection presentation (t-test, $T_{11} = 3.4276$, $P < 0.001$; Figure 1) and also the day following defection (t-test, $T_{11} = 6.084$, $P < 0.001$; Figure 1).

Decreased expression of nest behavior observed for males in the experimental treatment might result from differences in behavior by neighbor males between control and experimental treatments. We therefore compared defense behavior

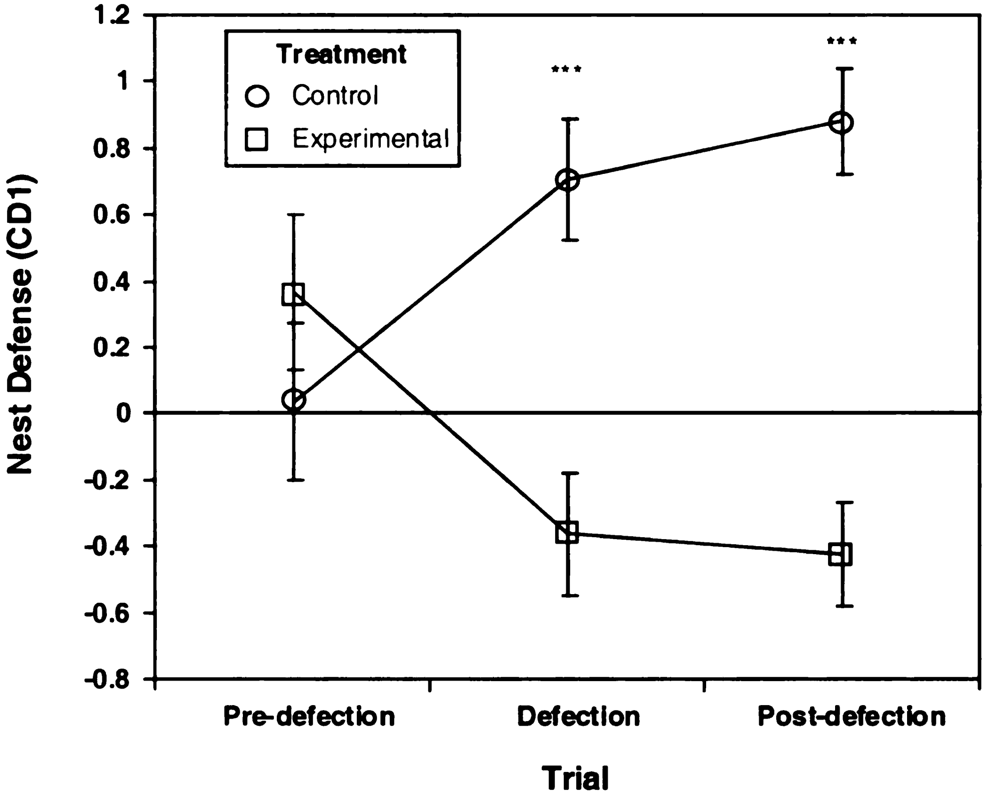

Figure 1. Nest defense by the focal male in response to treatment. Experimental treatments simulated defections by the neighbor male, control treatments allowed cooperative defense by both males. Error bars show ± 1SE. N= 6 for both treatments. *** $P < 0.001$.

of neighbor males between treatments in both pre- and post- defection presentations. We found no difference in defense behavior by neighbor males in response to the treatment or between trials (repeated measures ANOVA, treatment $F_{1,11} = 0.047$, N.S., trial $F_{1,11} = 0.882$, N.S., treatment * trial $F_{1,11} = 0.044$, N.S.; Figure 2). The results of the defection experiment therefore appear to result only from the response of the focal males to defection of their neighbor.

We found no difference in nest defense between a cuckolding male's defense at a nest on another territory and a randomly chosen neighbor who had not cuckolded that nest (paired t-test, $T_{24} = .654$, N.S.; Figure 3). It is possible that males are able to gain copulations with more than one female on a territory and only a few of those copulations result in fertilizations. We therefore compared average level of defense for all nests on a territory by a male that gained fertilizations on that territory and a randomly chosen neighbor that had not gained any fertilizations. There was again no difference in nest defense between males that had gained fertilizations on a territory and those that had not (paired t-test, $T_{20} = 0.323$, N.S.).

If females solicit copulations from several males in order to gain additional parental care in the form of increased nest defense, then nests with cuckolded young might receive more helpers than those without cuckolded young. We found a significant association between the number of males defending a crow and cuckoldry (nested ANOVA, territory $F_{13, 19} = 1.708$, N.S., cuckoldry

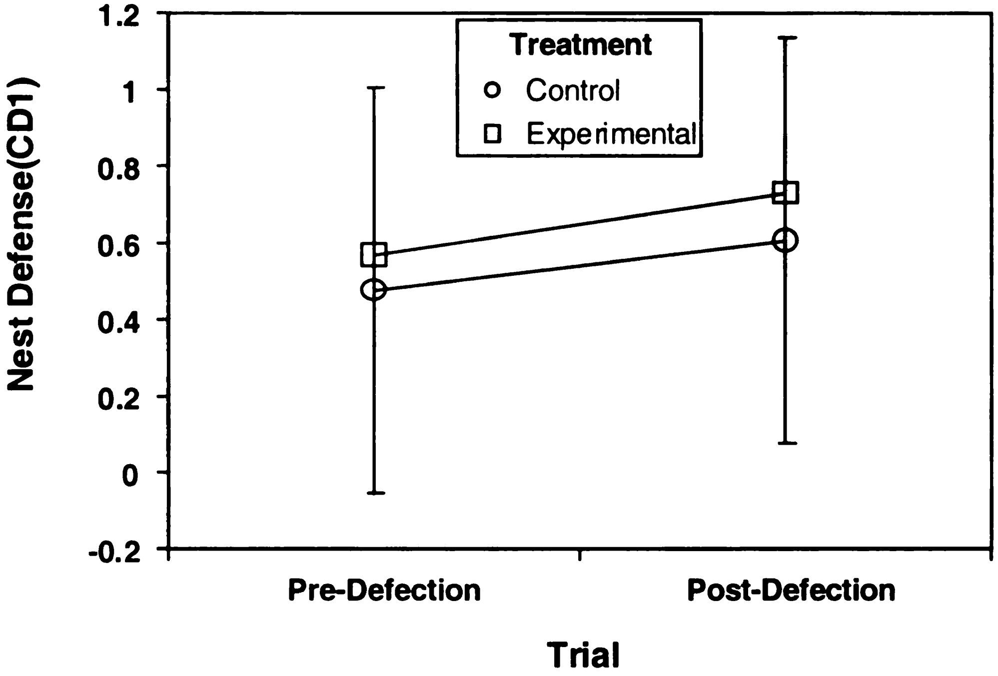

Figure 2. Nest defense behavior by neighbor males in pre- and post- defection trials in response to treatment. In experimental trials the neighbor male was held in a covered cage for the duration of the experiment. In control trials the neighbor was captured and released. Error bars show ± 1 SE. N = 6 for both treatments.

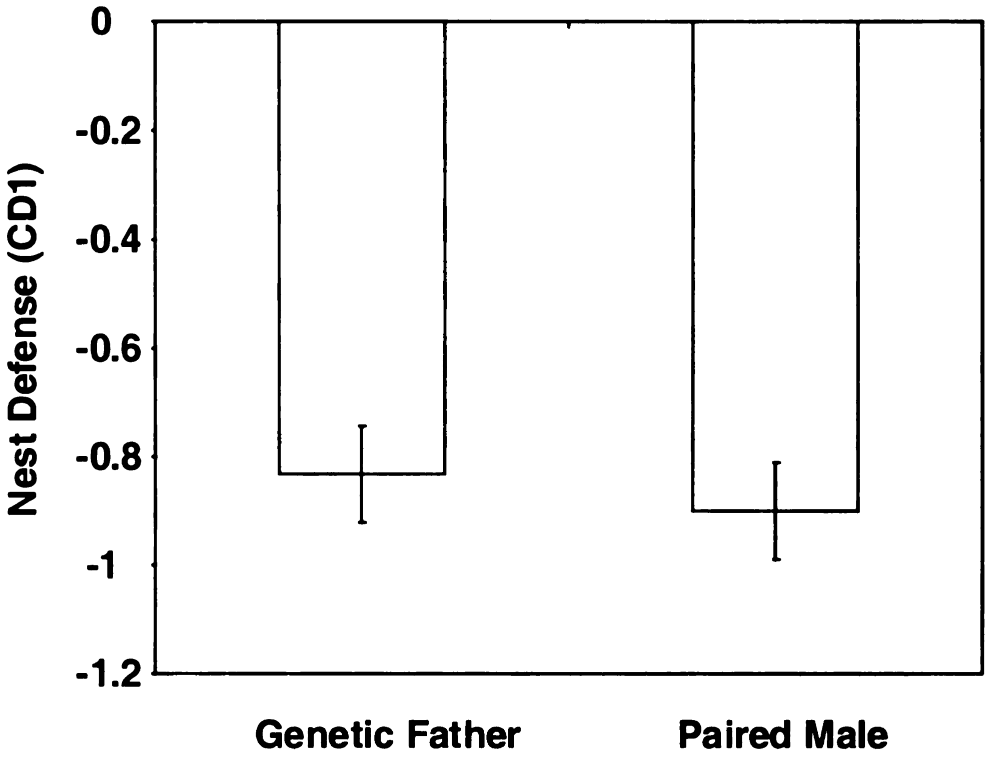

Figure 3. The level of nest defense by males at nests on other territories where they had EPF's and a randomly chosen male neighboring the same territory that was the same distance from the nest. N = 25. Error bars show ± 1 SE.

(territory) $F_{19, 32} = 2.498$, $P < 0.05$; Figure 4) the difference however is the opposite of the predicted direction. Nests without EPFs had more helpers than those with EPFs.

Territorial males may reduce their defense of nests on their territory if they are able to determine that the nest contains cuckolded offspring. We again used a nested analysis to account for the fact that males have multiple nests on each territory. Territorial males did not alter their level of nest defense in response to cuckoldry (nested ANOVA, territory $F_{13,19} = 1.659$, N.S., cuckoldry (territory) $F_{19,32} = 1.751$, N.S.; Figure 5).

DISCUSSION

We found that males cooperate in nest defense primarily as a form of reciprocal altruism. In simulated defections, males decreased defense against a nest predator when their neighbor appeared to defect by not defending, but increased defense in control trials. There was no association between nest defense and cuckoldry suggesting that mutualism plays little role in cooperative nest defense.

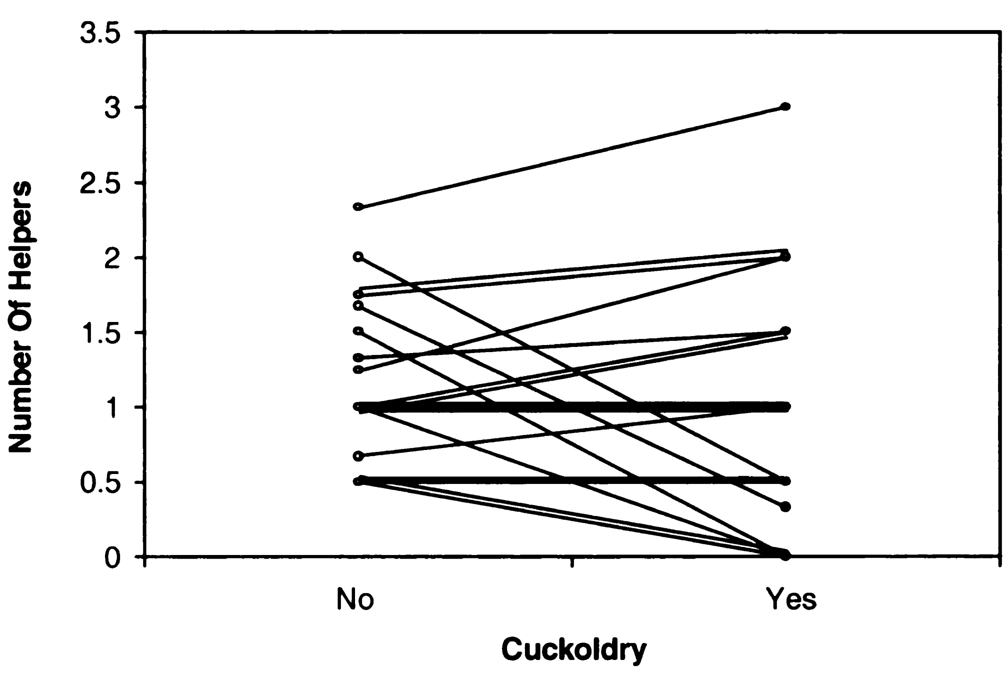

Figure 4. Average number of helpers at nests with cuckolded offspring compared to nests without cuckolded offspring. Each circle represents the average number of helpers at cuckolded and uncuckolded nests. Lines connect data from within a territory. N = 19.

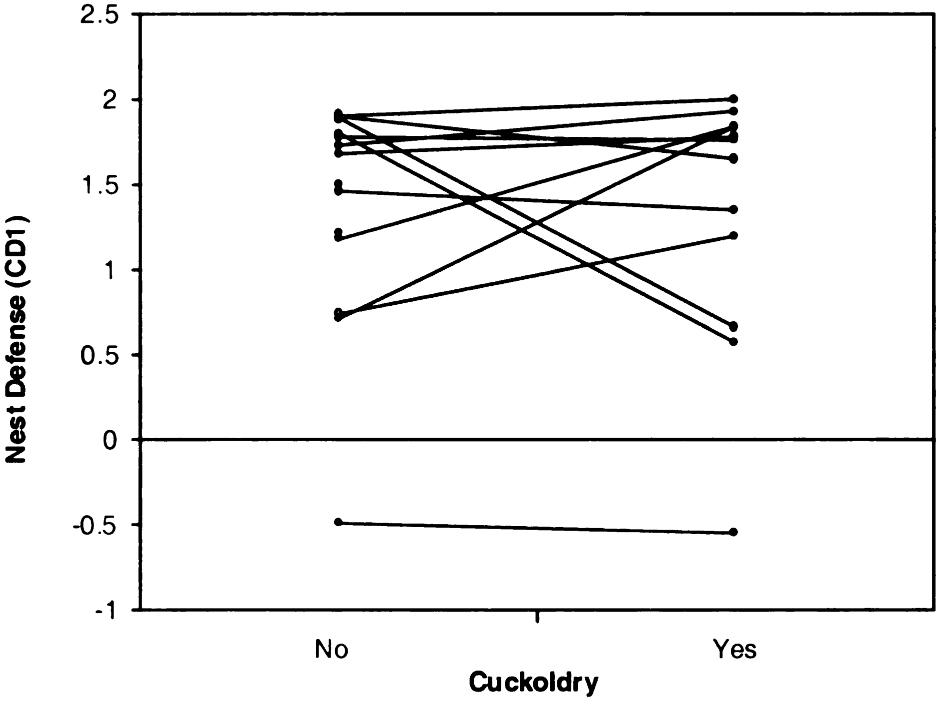

Figure 5. Nest defense by territorial males in response to cuckolded offspring in their nests. Circles represent average defense by the territorial male at cuckolded and uncuckolded nests. Lines connect data from within the same territory. N = 19

Nest Defense as TFT

If individuals engage in play TFT-like behavior as a form of cooperation then they should generally cooperate, but in response to defection, should retaliate by not cooperating. Additionally, long relationships enhance the advantage to individuals that use TFT-like strategies (Axelrod and Hamilton, 1981). Our study shows that male red-winged blackbirds reduce their level of defense when it appears their neighbor is not cooperating. Retaliation indicates TFT-like strategies and suggests that individuals that do not cooperate receive a short-term benefit (Rothstein and Pierotti, 1988). This is consistent with Beletsky and Orians' (1989) findings that males that had been neighbors in previous years suffered less from nest predation. Although they did not specifically test for TFT-like relationships they surmised that the reduced predation they observed resulted from cooperative nest defense. Unfortunately, in the year for which we have defense data, not enough males returned from the previous year to determine if familiar males were more likely to cooperate.

It could be argued that the reduction in defense we observed was in response to increased risk to the focal male when the defecting male did not participate in defense. By defending alone, the focal male was likely at greater risk of injury than when the neighbor aided in defense (Hamilton, 1971). Similar arguments have been proposed to explain apparent retaliatory behavior by guppies and sticklebacks in predator inspection. In these studies, guppies or sticklebacks approach a predatory fish and the fishes reflection in a mirror is used to simulate another fish that either approaches simultaneously with or lags behind the focal fish (Dugatkin, 1988; Milinski, 1987; Milinski et al., 1990). An individual's unwillingness to approach the predator, however, can be attributed to selfish herd like mutualism rather than tit-for tat-like behavior (Lazarus and Metcalfe, 1990; Masters and Waite, 1990). Although, subsequent experiments have further strengthened the case for TFT in predator inspection (Milinski, 1990; Milinski, 1992; Milinski, 1996) the initial criticisms are nonetheless potentially valid in this study.

It is unlikely increased risk to the focal male caused the results found in our study. Increased risk may have caused the focal male to decreases nest defense in the defection presentation, but it does not explain why males continued to show decreased nest defense in the post defection presentation. Especially since the neighbor male had returned and was defending normally by then. The only explanation therefore is that males reduced nest defense in the post presentation trial because they were retaliating in response to the perceived defection by their neighbor.

Males in control treatments increased their defense considerably between pre-defection presentations and defection and post-defection presentations. This is likely caused by repeated presentation of the crow over the course of a trial. Males may defend more aggressively with repeated exposure to models of nest predators because they learn that they have "successfully" driven it off with no negative consequences (Knight and Temple, 1986). This was an unavoidable artifact of our design, but does not influence the interpretation of our results since we presented the crow equally to both treatments. In fact, it makes the decrease in defense by males in the experimental treatment more convincing.

Nest Defense as Mutualism

Other studies have suggested that there is a mutualistic component to cooperative nest defense in red-winged blackbirds (Gray, 1997; Weatherhead et al., 1994). Both of these studies suggest that males help at other male's nests because they have sired offspring in those nests. Gray (1997) showed that males are more likely to defend at nests where they have cuckolded offspring and Weatherhead et al. (1994) show that nests that contain extra-pair offspring enjoy higher nesting success. This conforms to by-product mutualism because the helping males are most likely reducing nest predation for offspring of the territorial male as well as it's own.

In contrast to the studies outlined above, our study suggests that mutualism played little role in nest defense in our population. Males did not defend at nests where they had cuckolded offspring (Figure 3). Nor did cuckolded nests appear to attract more helping males than nests that had not been cuckolded (Figure 4). In fact, there was some tendency for nest that uncuckolded nests to attract more helping males. This result is surprising given that there would seem to be an obvious advantage to defending your own young from a nest predator. Territorial males were also unresponsive to levels of cuckoldry on their own territory and did not reduce their defense at cuckolded nests as expected (Figure 5). This again is contrary to results in other populations (Weatherhead et al., 1994).

Differing rates of cuckoldry cannot explain these differences. We found a similar rate of cuckoldry in our population as Gray, approximately 33% of offspring and 50% of nests were cuckolded, although this rate is somewhat higher than Weatherhead's population. Nor is it likely that males are not responsive to rates of cuckoldry. Other work in this population has shown that males react more aggressively to neighbors that have gained EPFs on their territory (Olendorf and Scribner, unpublished data).

Weatherhead (1995) found that in a population of red-winged blackbirds in Ontario, female nesting success did not increase with male familiarity. Beletsky and Orians (1989) found that familiarity enhanced breeding success in population of red-winged blackbirds in Washington. Weatherhead suggested that one possible reason for differences between his and Beletsky and Orians' was different density of red-winged blackbirds and nest predators.

In our study, site mammalian predators are rare because they are fenced out. Crows are common in the area and frequently forage at the pond facility when red-winged blackbirds are not nesting there. During the nesting season, however, crows are seldom seen in the pond facility. The crows remain in the area, because the are frequently heard in the trees approximately 200 m away and when the red-winged blackbirds leave after breeding, the crows return immediately. Possibly, early defense by the red-winged blackbirds had succeeded in reducing risks to predation and all the males (and females and offspring as well) were benefiting from low predation rates.

Simultaneous tests for reciprocal altruism and mutualism

As outlined above, there is evidence for considerable heterogeneity in the existence of cooperative nest defense. Other studies (Beletsky, 1989; Weatherhead, 1995) specifically addressed the relationship between familiarity among males and nest success or the relationship between extra-pair paternity and nest success (Gray, 1997; Weatherhead et al., 1994). Although the presumed mechanism among all these studies, was cooperative nest defense among males, the cooperation can result either from mutualism (Gray, 1997; Weatherhead et al., 1994) or reciprocal altruism (Beletsky, 1989; Weatherhead, 1995).

In this study, we tested for both mutualism and reciprocal altruism. We found evidence for reciprocal altruism (retaliation and TFT-like strategies) but not mutualism (no relationship between EPFs and nest defense). Our results contribute to growing evidence for heterogeneity in cooperative nest defense among red-winged blackbirds and suggest that in the future it would be valuable to test for both reciprocal altruism and mutualism in similar studies. It would be especially interesting to see if in populations where male nest defense varies in response to cuckoldry, there is also evidence for reciprocal altruism.

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CHAPTER 4:

THE EFFECT OF POPULATIONS STRUCTURE ON THE EVOLUTION OF COOPERATION AND COOPERATIVE STRATEGIES IN ARTIFICIAL POPULATIONS

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ABSTRACT

Cooperative populations usually achieve higher average fitness than uncooperative populations. Cooperative populations are susceptible to invasion by uncooperative individuals, however, because uncooperative individuals may potentially achieve higher fitness if the selective environment conforms to the prisoners' dilemma. Four general solutions to this problem have been proposed, group selection, kin selection, reciprocal altruism and by-product mutualism. These explanations only deal with the maintenance of cooperation but do not explain how cooperation can arise in a population of uncooperative individuals. Previous work has suggested that population structure can be instrumental in facilitating the evolution of cooperation. These studies, however, did not vary the degree of population structure, nor did they allow for a large array of possible strategies to evolve. In this study, I use genetic algorithms in a simulation of structured populations to determine the degree to which

population structure can affect the frequency of cooperation and the types of strategies that evolve. The simulations show that populations composed of small subpopulations achieved higher levels of cooperation. Populations with the smallest subpopulations evolved strategies that were almost unconditional cooperators, while slightly larger subpopulations evolved strategies similar to tit-for-tat. This study shows that differences in subpopulation structure are influence both the quantity and quality of cooperation that evolves in a population.

INTRODUCTION

The existence of cooperative behaviors in nature presents a problem for biologists. If a cooperative behavior imposes a cost on the individual exhibiting the behavior while at the same time benefiting another individual, natural selection should work to eliminate that behavior from the population. To persist in the population the cooperative individual must therefore achieve some benefit that compensates for the apparent loss of fitness. Explanations of how the benefit is realized fall into four general categories, group-selection (Wilson, 1975; Wynne-Edwards, 1965), kin-selection (Hamilton, 1963), reciprocal altruism (Trivers, 1971) and by-product mutualism (Connor, 1986). Each of these explanations makes certain assumptions about population structure and the pay-off structure so that cooperation is favored. In this study, I use simulations using populations of genetic algorithms to study the role of population structure in determining how cooperation evolves and what strategies result.

Two selective environments can lead to the evolution of cooperation, the Prisoner's Dilemma (PD) and mutualism. The PD was developed for use in the economic and social sciences by Flood and Dresher for the Rand Corporation and popularized by Von Neuman (1953). In the PD two players can make one of two choices, cooperate or defect. If both players cooperate then they both receive a high payoff (R). If both players choose to defect, then both players receive a low payoff (P). If one player defects while the other cooperates, than the defecting players gets the highest possible payoff (T), while the other payoff receives the lowest possible payoff (S). The pay-off structure therefore results in the inequality (T > R > P > S). This creates an interesting tension, if both players cooperate than they both do well. If, however, the one player is reasonably certain the other will cooperate, then he should defect. The other player should therefore also choose defect so as not to receive the lowest payoff. Therefore, the only stable equilibrium is mutual defection. Group selection, kin selection and reciprocal altruism alter the rules of this game so that cooperation can evolve in certain circumstances.

Mutualism results when the inequality described above is altered so that (R > T,S > P). Since cooperation always pays better than not cooperation, cooperation should easily evolve in.

Cooperation can evolve through group selection provided the rate of mutation is low, and the population is highly structured with limited migration among small subpopulations. Populations are assumed ancestrally selfish. Mutation gives rise to rare cooperative individuals and genetic drift allows some populations to become fixed for the cooperative trait. Cooperative populations enjoy higher fitness than selfish populations and produce more migrants. The greater number of migrants coming for cooperative subpopulations results in enhanced colonization of empty habitats (Wynne-Edwards, 1965) or increased migration to occupied habitats in some situations (Wilson, 1975). Over time cooperative subpopulations come to dominate selfish subpopulations.

Kin-selected cooperation results when individuals interact with related individuals. Any fitness costs associated with cooperative behaviors are compensated for by the increased fitness enjoyed by relatives (Hamilton, 1963). Lack of dispersal and small population size can cause related individuals to interact more often than would be expected otherwise. Kin-selection and group selection can therefor work together to favor cooperation.

If individuals have a great enough probability of playing the game again, the game is transformed into a new game known as the Iterated Prisoner's Dilemma (IPD). Because the players may play the game more than once, cooperative strategies can often outperform always defect (ALLD) by reciprocating altruistic acts (Trivers, 1971). By conducting a series of tournaments where people submitted strategies to play in the IPD, Axelrod (1980a; 1980b) ignited interest into effective strategies to play in the IPD. In the first tournament submitted strategies played against one another in a round robin tournament (Axelrod, 1980a). Much to his surprise tit-for-tat (TFT), one of the simplest strategies submitted won. TFT cooperates on the first move, and echoes the opponent's previous move thereafter. In a subsequent tournament where strategies competed in a more evolutionary context, TFT again did very well. However, several other strategies, most similar to TFT, also performed well (Axelrod, 1980b). Axelrod and Hamilton (1981) then went on to apply evolutionary game theory (Maynard Smith, 1982) to argue that TFT was an ESS strategy. Other studies have since shown that while TFT is very robust it is not strictly an ESS because other strategies can do as well as or possibly even slightly better than TFT in the IPD (Boyd and Lorberbaum, 1987; Dugatkin and Wilson, 1991; Nowak and Sigmund, 1993; Posch, 1999).

Despite the robustness of these strategies in pure populations, none of these strategies can easily invade a population of selfish individuals. When cooperators are rare in a population, they most often interact with selfish individuals. TFT for instance would cooperate on the first iteration of the game and receive the lowest possible payoff while the selfish individual would receive the highest payoff. Although it would defect thereafter, the initial loss of fitness relative to the selfish individual would prevent it from increasing in frequency through natural selection. If the population is structured and the individual subpopulations are small, genetic drift can allow the frequency of TFT can pass a threshold where it becomes favored by frequency dependent selection.

Following the example of Axelrod's (1980b, 1980 #35) tournaments many recent studies of the evolution of cooperation rely on simulations (e.g. Brauchli et al., 1999b; Brembs, 1996; Nowak and Sigmund, 1993; Posch, 1999; Sigmund and Nowak, 1998). Simulations have several advantages over analytical solutions when studying the evolution of cooperation. Analytical models can easily describe two competing strategies in a population, When additional strategies are added, however, analytical models become difficult or even impossible to solve. Simulation models overcome this difficulty by directly modeling the strategies as individual agents in a population. Individual agents interact based on their assigned strategies and reproduce according the fitness achieved through those interactions. More recently, genetic algorithms (GAs) are being used to model the evolution of cooperative behaviors (Sigmund, 1998).

GAs are essentially computer programs that give instructions on how an artificial agent should behave (Holland, 1975). Although initially developed by to by computer scientists to solve complex problems that cannot be solved analytically, they are increasingly used by biologists to study evolutionary processes (Mitchell, 1998). A large number of agents (individual variants of a GA) are created, each variant is a potential solution to a problem. The structure of the algorithm varies widely and is dependent on the nature of the problem. They can be as simple as a vector of number to be used as parameters to actual computer programs. In all cases, each GA is potential solution to the problem and the fitness of each GA is determined by its ability to solve the problem. The most successful GAs either go through differential reproduction or differential death so that the GAs that best solve the problem increase in frequency. A fraction of the agents are mutated altering their algorithm and the process is repeated. Mutations allow the testing of new potential solutions that are similar to already successful solutions and help to fully explore the adaptive landscape. The similarity of these techniques to biological evolution is not accidental. Computer scientists purposely copied nature reasoning that evolution is a problem solving system using individual life forms as potential solutions. Formal studies into effective mechanisms for selecting good algorithms in fact suggest that mechanisms that most closely model biology are among the most effective at finding good solutions to complex problems.

Genetic algorithms are a natural tool to use to study the evolution of behavior provided an adequate system can be devised to generate the behaviors of interest. Several recent studies have used genetic algorithms to show that a certain strategies is an effective solution to the PD (e.g. Nowak and Sigmund, 1993; Posch, 1999). These algorithms, however, are designed to easily code for the strategy under study. All of these studies assume that there is no need to have a memory more than three interactions in the past.

Several simulation studies have also shown that population structure is an important factor in the evolution of cooperation (Brauchli et al., 1999a; Killingback and Doebeli, 1998; Mitteldorf and Wilson, 2000). These studies, however, only use at most a handful of known strategies to study this relationship. In this study, I develop a scheme for generating algorithms that can code for a large variety of strategies, including most commonly discussed strategies. I addition, there is virtually no limit on length of memory. Using structured, populations I will determine under which conditions of population size and dispersal the evolution of cooperation is favored. Additionally, I will determine if population structure can influence the types of strategies that evolve. For instance, always cooperate might evolve under circumstances favorable to group selection, but TFT might evolve in somewhat less structured populations.

THE MODEL

The Genetic Code

The model presented in this study uses genetic algorithms to determine the strategies of the individuals. The principal behind this system of generating strategies was to create simple mechanism that would easily and succinctly code for many commonly discussed strategies in the literature as well as a great number of strategies that may not have been previously studied. In addition, this system allows for easy interpretation of the "DNA" strings to determine the strategies for which they code.

The basic units of the system are a set of five characters (**c**, **d**, **:**, **/**, *****) arrayed along a one-dimensional array, the "chromosome" (Figure 1). The structure of the chromosome consists of a series of "genes" along the chromosome. Each

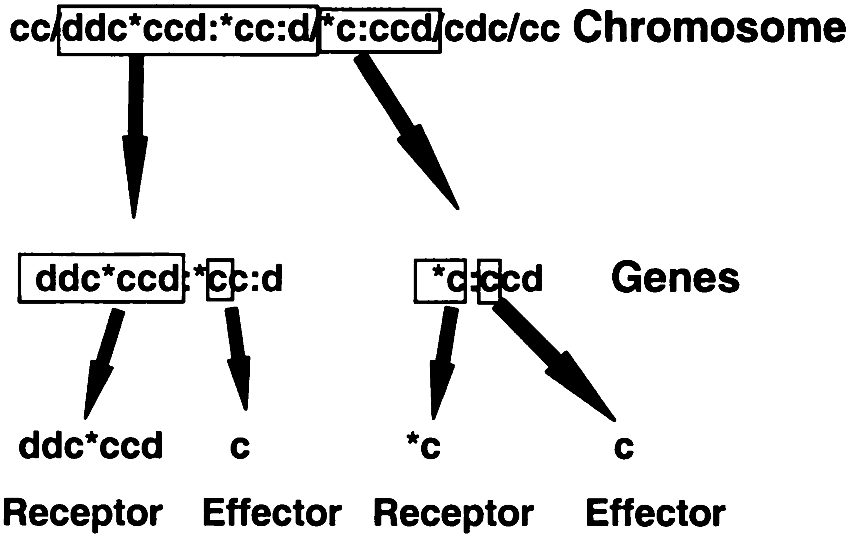

Figure 1. The structure of the genetic algorithm. A chromosome is constructed of an array of characters. Individual genes within a chromosome delineated by slashes. Each gene consists of a receptor and effector portion. The receptor starts at the left slash and continues to the first colon. The receptor is a pattern to be matched by an opponent's behavioral history. The **c**'s and **d**'s correspond to previous plays, the most recent on the right. Asterisks (*) match any combination of characters or no characters. The receptor is the first **c** or **d** to the right of a colon, the remainder of the string is ignored until the next functional gene is encountered.

gene starts at a slash and ends at the next slash. The structure of each gene consists of a receptor and an effector. The receptor consisted of a series of **c**'s, **d**'s and *'s starting from the left most slash to the first colon. The **c**'s and **d**'s reflect the behavioral history of an opponent, the most recent interactions being on the right. The asterisks can stand for any number of characters. For example, a receptor of **d*c** would match any history where the opponent defected on the first move and cooperated on the most recent move, any intervening interactions are ignored. The effector portion of a gene consists simply of the first **c** or **d** to the right of the first colon. A single gene, therefor, consists of a pattern on the left of a colon that, if matched, elicits a response specified on the right of the colon (Figure 1). The set of genes on a chromosome, reading from left to right, specifies an individual's repertoire of stimulus/response sets or its strategy. In many instances, genes are not interpretable in this system and uninterpretable genes are ignored. If more than one pattern matches the opponent's behavioral history, the first matching pattern from the left is used. If no patterns match, the individual plays defect. Setting the default response to defect is analogous to the hypothesized primitive condition of defection in animals.

Strategies such as tit-for-tat, always cooperate and always defect are easily written and easily recognized using this system (Table I). Additionally, a wide variety of other strategies can also be generated at random. This system does ignore the individuals own behavior and the payoffs received. It therefore

Table I. Examples of chromosomes along with the associated strategies. The names of commonly discussed strategies are also show.

Commonly Discussed Strategies

Chromosome	Receptor : Effector Pairs	Strategy	Abbreviation
/*:d/	(* : d)	Always Defect	ALLD
/*:c/	(* : c)	Always Cooperate	ALLC
/*d:d/*:c/	(*d : d),(* : c)	Tit-for-tat	TFT
/*dd:d/*:c/	(*dd : d),(* : c)	Generous TFT	GTFT

Randomly Generated Chromosomes

Chromosome	Receptor : Effector Pairs
*d:d	(* : d)
*///:/dd/cd:*d*d///cdc:d/cdd	(cd: d),(cdc : d),(* : d)
d*/d:::d*/:*d*::	(d : d),(* : d)
c:*c*::cd:::c*d**d//* d/://c*///d/*dcc*d::cd:/:	(*dcc*d : c),(* : d)
:/: d/ d:*/ c*:cc/ ccdc:dc*cdcc*/ dcdd*/ cd	(c*d c),(ccdc : d),(* :d)

cannot code for strategies such as Pavlov (Posch, 1999) where an individual must be aware of its own history and the payoff structure as well as its' opponent's play history. Such strategies, however, require additional cognitive skills on the part of biological animals such as memory of both its own and it's opponents behavioral history or knowledge of the payoff structure. This results in an analogous increase in memory usage by the agents. One of the goals of this study is to determine the simplest conditions under which cooperation might evolve. It would be interesting to study such things in the future but it is beyond the scope of this study.

The Simulation

All populations in this study were composed subpopulations arranged in a 10 X 10 array. I varied population size within subpopulations (carrying capacity) and dispersal between subpopulations. Populations were first initialized by either randomly generating 100 character chromosomes for each individual or supplying all individuals with a specified strategy.

Two individuals were selected randomly with replacement from within a sub-population to interact. Each interaction consisted of a number of bouts. After each bout, a pseudo-random number was generated. If the number was less than 1/15, the interaction ended and new individuals were drawn. The process was repeated N times (N = subpopulation size).

Its chromosome and the opponent's behavior determined each individual's behavior. The fitness for each interaction was determined using the defined pay-off structure (i.e. Prisoner's Dilemma). The mean fitness per interaction was used to select among strategies to standardize for interaction length.

After interactions were complete, individuals were selected to were subjected mutation and crossing-over. I set the mutation rate to be 1 X 10^{-6} mutations/character. This rate is within the observed mutation rate in natural populations (Nei, 1987). This rate of mutation also resulted in good rates of evolution when simulating simple problems such as evolving a population of pure defectors to pure cooperators in a mutualistic environment with no crossing-over. I allowed substitution, insertion and deletion mutations to occur. I set the rate of crossing-over to be 10% of the population in a generation. Higher rates of crossing-over appeared to hinder the ability of a population to find the fitness peak in the simple problem outlined above.

After mutation and crossing over, individuals reproduced based on their relative fitness within each sub-population (soft selection). Individuals then dispersed from subpopulations. The number of dispersing individuals was equal to the surplus number of agents in the subpopulation. I varied dispersal rate varied by varying mortality among dispersers from 0 to 100%. In other words, if a subpopulation had a carrying capacity of 10, and after reproduction contained

15 agents, 5 agents would attempt to disperse. If the dispersal rate were 0.1, then on average only 0.5 agents would successfully disperse. If a sub-population was still over carrying capacity following dispersal, individuals were removed at random until carrying capacity was reached (density independent mortality). Each individual's fitness was reset to the initial value and the process was repeated for the specified number of generations.

Experimental Design

To determine the affect of population structure on the evolution of cooperation, I varied subpopulation size (10, 25, 50, 100) and dispersal rate (0, 0.0001, 0.001, 0.01, 0.1, 1) in a fully crossed design. Ten runs of 500 generations were completed for each combination of parameters for a total of 240 runs.

I used the frequency of cooperative interactions as a measure of cooperation. I also determined the most commonly used strategies in each setting. With randomly generated chromosomes, there was typically a wide diversity of chromosomes at the end of each simulation. There was considerably less variation in strategies although the number of different strategies was still formidable. Much of that variation however, resulted from similar strategies that differed in a single gene that had little impact on the function of the agent. The simplest form of TFT is coded by; **/*d:d/*:c/**. Addition of a new gene such as **/cddcd:c/*d:d/*:c/** would not alter the behavior of the agent because the receptor of the additional gene is so specific. To facilitate analysis, I therefore grouped similar strategies together. Granted, this is a subjective process, so I was conservative in grouping strategies together.

Three different combinations of initial strategy compositions and selective environments were used. I first ran randomly generated individuals in a mutualistic environment to ensure that cooperation could evolve in each setting. I also ran pure TFT populations in a PD environment to establish that TFT was resistant to invasion. I then ran randomly generated individuals in a PD environment to determine how population structure affected the evolution of cooperation from selfish population.

RESULTS

Randomly Generated Agents in a mutualistic environment

All populations achieved high levels of cooperation in a mutualistic environment, reaching 99% cooperation. Despite the generally higher levels of cooperation achieved in the mutualistic environment, there was a significant affect of subpopulation size but there was no effect of dispersal (multiple regression, dispersal $T = 0.0002$, N.S., subpopulation size $T = -13.736$, $P < 0.001$, $R^2 = 0.465$, $F_{2,222} = 96.621$, $P < 0.001$; Figure 2 & 3). The highest levels of

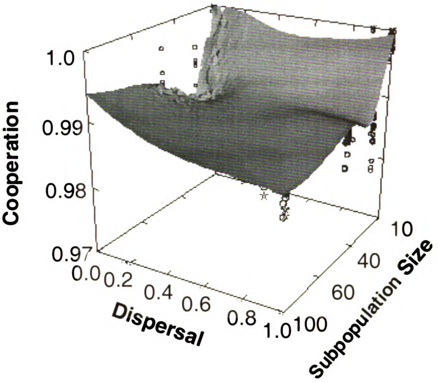

Figure 2. Level of cooperation achieved after 500 generation by randomly generated populations in a mutualistic environment varying dispersal and subpopulation size. The surface was fit using DWLS smoothing.

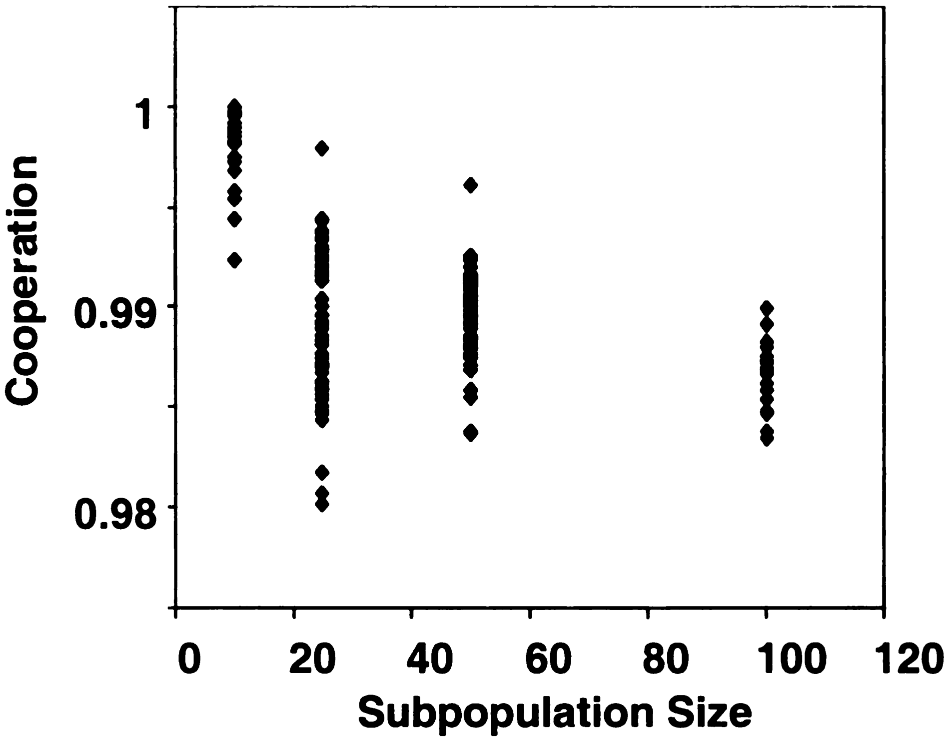

Figure 6. The level of coopration achieved in all populations in response to subpopulations size in mutualistic environments.

cooperation were found in populations composed of subpopulations of 10 agents and decreased slightly as subpopulation size increased. The most common strategies in all runs were effectively equivalent to ALLC.

Pure TFT in PD environments

TFT proved to be resistant to invasion by other strategies in all environments (Figure 4). There was a very slight dip in levels of cooperation in populations with low dispersal and high population size but the change was very small. There appeared to be little increase in the frequency of other strategies.

Randomly Generated Agents in the PD environment

When starting with randomly generated chromosomes, the initial level of cooperation within a population was approximately 2% of all interactions. After 500 generations, the average level of cooperation among all runs was 13%. Subpopulation size was the most important factor influencing the evolution of cooperation while dispersal had no affect (regression, dispersal $T = -0.498$, N.S., population size $T = --8.008$, $P < 0.001$, $R^2 = 0.214$, $F_{2,237} = 96.621$, $P < 0.0001$; Figure 5 & 6). Populations consisting of subpopulations of 10 agents had the highest level of cooperation at 40% of interactions. Cooperation decreased with increasing subpopulation size until at 100 individuals per

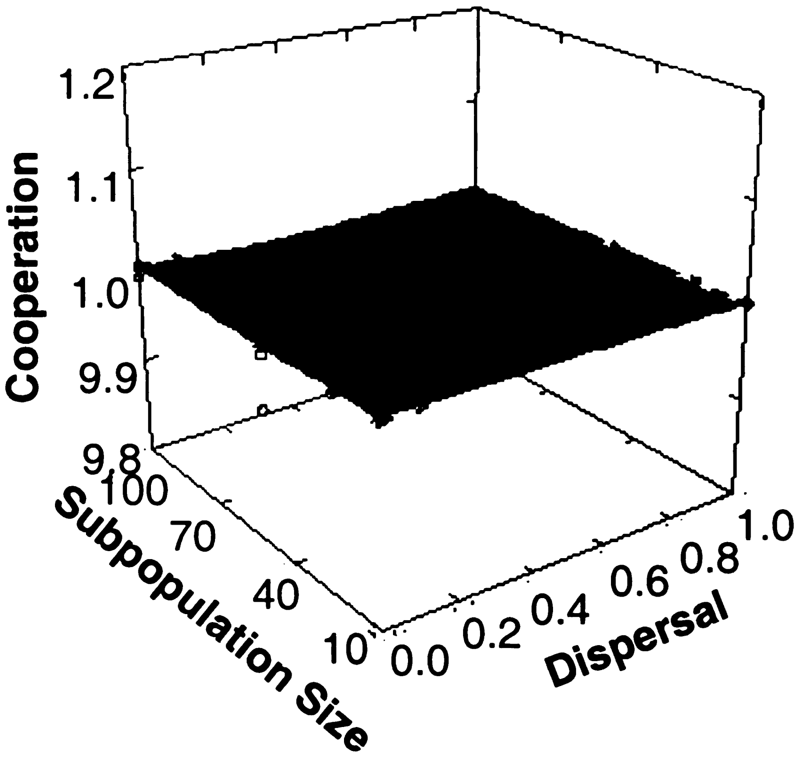

Figure 4. Level of cooperation achieved after 500 generation by initially pure TFT populations in a PD environment varying dispersal and subpopulation size. The surface was fit using DWLS smoothing.

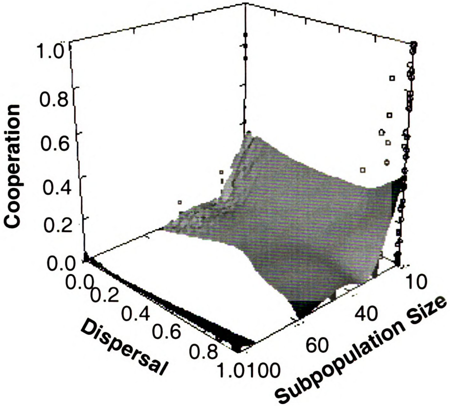

Figure 5. Level of cooperation achieved after 500 generation by randomly generated populations in a PD environment varying dispersal and subpopulation size. The surface was fit using DWLS smoothing.

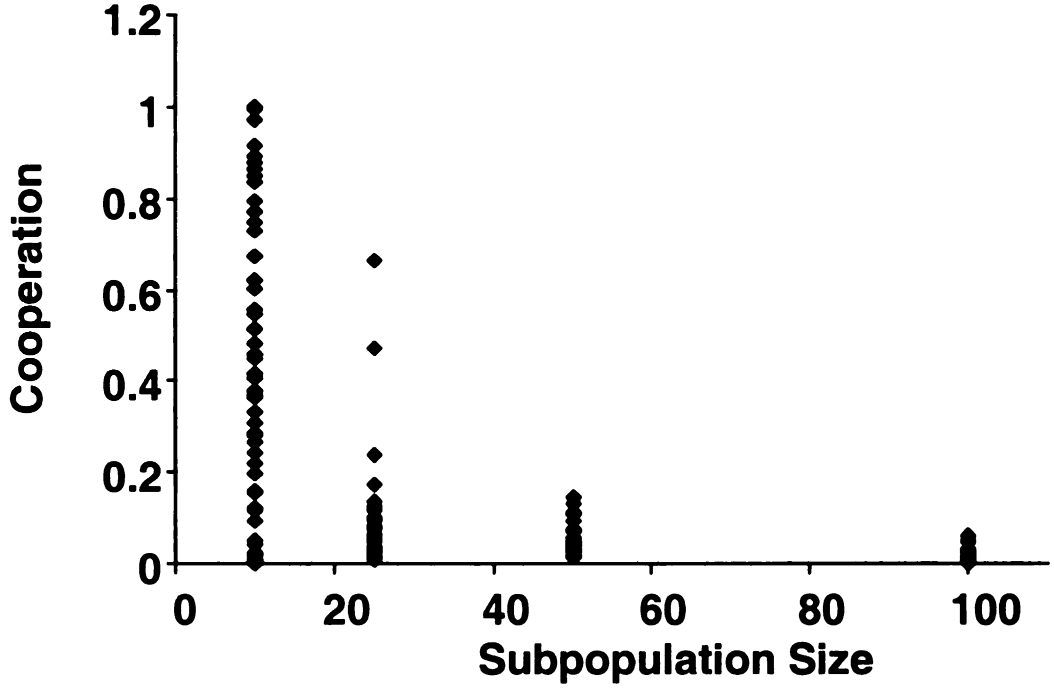

Figure 6. Level of cooperation in relation to subpopulation size. All runs are included regardless of dispersal.

subpopulation, the levels of cooperation was identical to randomly generated agents.

The combination of dispersal and subpopulation size influenced the type of strategies that evolved (Figure 4). When dispersal and populations size were low, the most common strategies were similar to always cooperate strategy (ALLC) but often differed in one aspect. These strategies always cooperated unless the opponent defected on the first play, in which case it always defected (ONED). TFT evolved most commonly in intermediate levels of dispersal and subpopulation size (Figure 4). Always defect (ALLD) was the most common strategy to evolve in populations defined by large subpopulations and high dispersal.

DISCUSSION

The simulations presented in this study verify the broad prediction that in PD environments, population structure can facilitate the evolution of cooperation both through reciprocal altruism and group selection. We also show that at least in the short term, novel cooperative strategies can evolve. The simulations also corroborate the expectation that the TFT strategy is a robust strategy, not easily invaded by other populations.

Dispersal				
1	TFT	ALLD/TFT	ALLD	ALLD
0.1	TFT	TFT	ALLD	ALLD
0.01	ONED	TFT	ALLD	ALLD
0.001	ONED	TFT/ONED	ALLD	ALLD
0.0001	ALLC	ONED	ALLD	ALLD
0	ONED	ALLC	ALLD	ALLD
	10	25	50	100

Population Size

Figure 7. The effect of population structure on the types of strategies that evolved in the populations is shown. TFT = tit-for-tat, ALLD = always defect, ALLC = always cooperate, ONED = test for defection on the first play only.

Several recent studies have demonstrated that population structure can facilitate the evolution of cooperation (Brauchli et al., 1999a; Killingback and Doebeli, 1998; Mitteldorf and Wilson, 2000). These studies corroborate earlier analytical studies that predict such a relationship (Axelrod and Hamilton, 1981; Wilson, 1975). Small subpopulations allow genetic drift to occur so that strategies such as TFT can reach a critical threshold where frequency dependent selection begins to favor them (Boorman and Levitt, 1973). It has also been argued that population structure can facilitate the evolution of ALLC-like strategies via group selection (Wilson, 1977; Wynne-Edwards, 1965).

In this study, subpopulation size appears to be the dominant force influencing the rate at which populations evolve cooperative behaviors. This is a somewhat surprising result since dispersal determines how distinct subpopulations are. It is likely that this result is an artifact of the simulation. Even in runs where dispersal was set at one, individuals were only able to move to neighboring demes. This limited amount of dispersal may not have been able to disrupt local population structure. If individuals had dispersed more widely, dispersal would probably have had a greater effect. Nonetheless, this study supports the hypothesis that population supports the evolution of both group selected cooperation and reciprocal altruism.

Kin selection may also have played a role in the evolution of cooperation in these simulations. Several generations of breeding within small populations leads to inbreeding. The greater than average relatedness among the individuals in a subpopulation suggests that altruism is probably always directed towards kin. This makes teasing apart the forces of kin selection and group selection difficult since inbreeding will always occur in small populations. There is one important difference between kin selection, as described by Hamilton, and kin selection that might occur in small populations. In classical kin selection individuals take advantage of heterogeneity of relatedness within a population (Hamilton, 1963). Kin selection in a small population there may be little heterogeneity of relatedness among individuals. It may therefore be more appropriate to refer to the evolution of cooperation in small populations to be evolving as a result of group selection.

This study also suggests that group selection can favor cooperation in conditions where subpopulations are very small and distinct. However, one novel strategy evolved, ONED. This strategy was the same as ALLC except that reverted to always defect if it's opponent defected on the first move. This strategy has the ability to detect ALLD early on much like TFT. However, it has two serious weaknesses. First, it only checks for defection on the first move. Strategies that cooperated on the first move and defected afterwards would have a strong advantage against ONED. Second, it does not forgive so that it may lose the opportunity to cooperate with more cooperative strategies that happen to defect on the first move. It is likely that this is a transitory strategy, and possibly is a step on the way to evolving a true TFT strategy. All of our runs lasted for only 500 generations. Longer runs would determine the ability of ONED to persist.

Brauchli et al. (1999a) found similar results. They ran simulations of all stochastic strategies with one round memories. Their simulations show, as in my simulations, that spatial structure can not only influence the level of cooperation but also the strategies used the evolve. In addition, their spatially structured populations were invaded by transient strategies that facilitated the establishment of more robust strategies such as PAVLOV (win stay, lose shift) or TFT.

Nowak & Sigmund (1993) show that the Pavlov strategy cannot invade a population of defectors without first without another cooperative strategy such as TFT establishing itself first. These results suggest that ONED in our populations is quite possibly a transient strategy, that could possibly facilitate the evolution of other more robust cooperative strategies.

One problem with structured populations where a variety of different populations can coexist is noise. Although two different strategies might be cooperative, differences between the strategies might lead to mutual defection. Brauchli et al. (1999b) found that generous strategies were often favored under these circumstances. Their model, however, used stochastic strategies. Individuals could be forgiving by reducing the probability of defecting in response to defecting. Grim (1995) and Sigmund & Nowak (1992), however, also found that spatial structure favored generous strategies in reactive strategies.

Generosity did not develop in my simulations. The most likely explanation for this is the short length of the simulations. Although high levels of cooperation were achieved under a variety of conditions, there may still be more robust strategies that had yet to arise. It is also possible, however, that the potentially long memory used in my simulations allowed strategies to forgo the potential cost of generosity.

The results of this study conform to the results from other studies. Spatial structure enhanced the evolution of cooperation, although I found that subpopulation size, but not subpopulation structure contributed to the evolution of cooperation. The simulations presented here extend previous studies by showing that not only does spatial structure facilitate the cooperation of cooperation, but that varying levels of population structure altars the trajectory and possibly the final composition of strategies in the population.

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