

1 SPOILS OF WAR AND PEACE: ENEMY ADOPTION AND QUEEN-RIGHT COLONY
2 FUSION FOLLOW COSTLY INTRASPECIFIC CONFLICT IN ACACIA ANTS

3
4 Intraspecific conflict over vital limited resources can lead to costly fights. How
5 winners compensate for costs and minimize the threat of Pyrrhic victory is not
6 well known. This study tracked the outcomes of experimentally induced field
7 conflicts between highly territorial Acacia ant *Crematogaster mimosae* colonies
8 using molecular genetics, and discovered that fatal fights significantly decrease
9 within-colony worker relatedness. We find that reduced relatedness can be
10 explained by colonies increasing worker number via 1) non-kin enemy adoption
11 or 2) queen-right colony fusion. We hypothesize that incorporating non-kin
12 enemies can speed recovery from conflict when resource defense is paramount.
13 In the case of queen-right colony fusion, territorial defense benefits could
14 outweigh fitness costs. We provide evidence that winners of colony fights have
15 reduced worker forces to defend larger territories. Field assays indicate that post-
16 fight colonies are more vulnerable to heavy browsing of host trees by mega-
17 herbivores and takeover by competitors following conflict. We discuss the
18 implications of our findings for ant colony cohesion and recognition systems.

19

20 KEYWORDS *Crematogaster*, *Acacia drepanolobium*, *conflict costs*, *territorial*
21 *aggression*, *worker relatedness*, *intraspecific slavery*, *colony fusion*, *Acacia ant*,
22 *Pyrrhic victory*.

23

24 Intraspecific conflict over resources can be costly. The evolution of assessment
25 systems enables many adult competitors to settle disputes with minimal physical

26 or energetic escalation [1,2] Still, intense combat does occur over particularly
27 limited and valuable resources (e.g. mates and territory, but rarely food) [3–5].
28 Victory in such high-stakes battles can be decided through elaborate displays, or
29 by violent contests [2] that continue until one opponent concedes from
30 exhaustion or injury, or dies (wars of attrition [6]; ‘desperado effect’ [7]).

31 Winners benefit from defeating opponents by gaining access to contested
32 resources. In addition, empirical studies widely document a positive feedback
33 between successful fighting experiences and probability of victory in future
34 contests, termed ‘the winner effect’ [8]. Yet by outlasting or dispatching
35 conspecific contestants, winners are also theorized to accrue costs associated
36 with escalation, including opportunity costs and loss of resource holding capacity
37 [9,10]. As a result, winners may experience a diminished ability to defend
38 themselves from predators and parasites, or to protect their gains in subsequent
39 contests [11,12]. This possibility, that winners suffer increased vulnerability after
40 engaging in fights, should be an important factor affecting potential costs and
41 benefits of engaging or continuing in conspecific fights [13]; yet experimental
42 tests are rare [5,14]. Furthermore, whether and how winners respond
43 behaviorally to cope with fight costs and facilitate recovery remains largely
44 unexplored. Here we investigate whether winners experience a window of
45 vulnerability following costly fights, and if so how they might compensate to
46 speed recovery from conflict.

47 Ants are compelling model organisms for this investigation for two main
48 reasons. First, violent fights over territory are common [15]. Intruders are grasped

49 or stung by defending workers, which often leads to dismemberment or death for
50 both combatants. Second, the impact of colony conflicts to winners can be
51 quantified in discrete units (i.e. individual workers killed). Most ant colonies
52 exhibit reproductive division of labor where workers forgo direct fitness to rear
53 immature workers and reproductives produced by the fertile queen(s). As a
54 consequence of this 'super-organism' arrangement, loss of the queen(s)
55 ultimately results in colony death [16]. Death of individual workers, however,
56 need not be fatal to colonies. Instead, these losses can be viewed as the costs of
57 conflict. Because colony size underlies victory in many ant species, significant
58 reduction in worker number likely affects a winner colony's ability to protect nest
59 space and territory after fights. Hence, quantification of worker deaths is directly
60 relevant to colony condition.

61 Do winners have tactics to recover from deficits incurred as a result of
62 costly fighting? Models predict that protracted and dangerous fighting most often
63 occurs when reproductive success or survival is at stake [4]. The mates or
64 security (e.g. burrows, nest space etc.) that are the payoff of fight success cannot
65 themselves be used to rebuild the winner's physical condition post-conflict. One
66 solution to rapidly replenish spent reserves can be cannibalization of defeated
67 contestants. Some spiders, squirrels, moths, owls, and ants consume
68 conspecifics after mortal combat for territory or shelter [17–19]. An alternative
69 path to recovery uniquely available to social insects is rebuilding colony size by
70 adopting a loser colony's surviving brood and/or workers [20]. In doing so, energy
71 lost in tissue conversion via consumption could be avoided, and time until viable

72 workers are present is reduced (possibly even approaching zero). The challenge
73 to this tactic is overcoming nest-mate recognition systems. Although often viewed
74 as fortresses of cooperative relatives, ant colonies can be permeable to non-kin.
75 Social and nest parasites invade host nests [21], unrelated queens found nests
76 cooperatively [22], heterospecific colonies share nests [23], queenless colonies
77 fuse [24] and some species, including invasive fire ants, reciprocally raid
78 neighboring colonies for brood, creating genetically blended colonies [25].
79 Mechanisms enabling tolerance for non-kin within nests include host chemistry
80 mimicry by usurpers, weak discrimination, recognition errors, signal mixing and
81 environmental modification of recognition cues [24,26]. In the latter case, even
82 contact with nest material can alter aggression patterns in ants [27]. Taken
83 together there appears to be a strong possibility that individuals from defeated
84 colonies can themselves become 'spoils of war', incorporated into post-conflict
85 winner colonies regardless of relatedness to the usurping colony.

86 Through a series of field manipulations with the African Acacia-ant
87 *Crematogaster mimosae* colonies we tested the hypotheses that intraspecific
88 conflict for nest space on *Acacia drepanolobium* trees results in significant
89 casualties for victor colonies and following these colony depletions, winners are
90 less able to defend host trees against herbivores and competitors. We also use
91 molecular markers to assess the outcome of conspecific fights and examine the
92 impact of fights on the genetic composition of colonies. We predicted that
93 territorial battles between conspecifics result in complete colony takeovers, and

94 that post-conflict winner workforces are built back in part through the adoption of
95 their non-kin enemies.

96

97 METHODS

98 This study was conducted from July 2011 to March 2012 at the Mpala
99 Research Centre in Laikipia, Kenya (37°53' E, 0°17' N). There as in many East
100 African savannas, colonies of Acacia-ant *Crematogaster mimosae* battle
101 elephants, giraffe, baboons, insect herbivores, conspecific and heterospecific
102 Acacia-ant competitors (*C. nigriceps*, *C. sjostedi* and *Tetraponera penzigi*) to
103 protect or maintain sole control of *Acacia drepanolobium* trees with which they
104 are obligate mutualists [28]. Colonies gain additional domatia (swollen thorn
105 nesting spaces) by initiating aggressive inter- and intra-specific wars to displace
106 neighboring colonies from their host plants [28]. Territorial battles also occur
107 when *A. drepanolobium* canopies grow together and when elephants topple trees
108 into one another [29]. In high-density, monospecific stands of *A. drepanolobium*
109 where >99% of trees are occupied by ants, up to 7.5% of resident colonies may
110 lose host trees to other ant species over a 6-month period [30]. *C. mimosae*
111 colonies are numerically dominant in the system (inhabiting 52% of trees [31]),
112 suggesting substantial intraspecific conflict and turnover within this species,
113 though exact rates are unknown.

114 Defeated colonies cede not only valuable domatia, but also their surviving
115 immature and possibly even mature workers [20]. In this widely studied
116 mutualism, there is no evidence of mixed species *Crematogaster* colonies

117 forming after interspecific fighting. Striking morphological differences would make
118 the presence of such colonies easy to observe. By contrast, in conspecific
119 conflicts, the identity of winners and losers cannot be visually detected. To
120 determine the consequences of conspecific conflict in this species we induced
121 battles between neighboring *C. mimosae* colonies and used genes and behavior
122 to track the consequences.

123 Experimental Colony Selection

124 Mature *Crematogaster mimosae* colonies can be small and restricted to single
125 host trees or form large multi-tree clusters with several queens and hundreds of
126 thousands of individuals [30,32]. To ensure that colonies would fight in
127 experimental battles and not retreat to auxiliary host trees, we chose only
128 colonies inhabiting single trees with basal diameter of 32-68 mm ($X + SE =$
129 45.4 ± 1.31). Colonies on trees of this size ranged from an estimated 2,697 to
130 9,870 workers ($X + SE = 4999 \pm 255$, calculated as domatia number * mean
131 number of workers per gall, 68.5) [28].

132 Focal colony trees were all located close enough together that the canopies
133 could be physically conjoined.

134 Single tree colonies were identified using reciprocal transplants of individual
135 workers and watching for aggressive interactions with resident ants [28,33].

136 Latex gloves washed with 95% ethanol prevented between-trial chemical
137 contamination of individuals. To aid observation of fast moving transplanted
138 workers, we applied florescent powder (Day-Glo Color Corp., Cleveland, OH) to
139 the thorax of trial ants. We treated individuals of the resident colony in the same
140 manner to act as procedural controls. If non-resident ants were quickly attacked

141 (but resident controls were not) we inferred that trees belonged to separate
142 colonies [28]. We repeated this method on neighboring trees up to 8m away to
143 confirm experimental colony monodomy . Each colony within the pair was
144 identified with metal tags as either A or B with a shared Fight ID number (Table
145 1).

146 *Manipulations*

147 Between 14-July and 04-August 2011, we induced fights between colonies
148 by tying the canopies of the two experimental trees together with wire. *Acacia*
149 *drepanolobium* stems are flexible and tolerated bending. Canopies remained
150 connected for 8 months after fights.

151 Immediately prior to fights we collected three healthy domatia filled with live
152 workers and brood from each tree. These ants were kept contained and isolated
153 in the lab, and fed on a diet of sugar water and tuna. To determine the identity of
154 the winning colony, live individuals ($N = 2-4$ from each of the pre-fight A and B
155 colonies) were returned to the field and placed on the main stems of both trees
156 on day 6 after fights and observed in the manner of reciprocal transplants
157 described above. Winner and loser colonies were determined by the combined
158 outcome of these behavioral experiments and molecular genetics (below).

159 *Costs of Fighting to Winners*

160 During territorial battles, workers from each colony engage in fights to the
161 death, with larger colonies the more likely victor [28]. Colony fights are expected
162 to produce heavy winner casualties. To quantify these costs we placed large
163 plastic tarps secured at ground level between some paired fight trees ($N = 7$) and
164 collected workers that fell from host plants when canopies were experimentally

165 joined and fighting commenced. Dead and injured workers on tarps were
166 collected every 24 hours (if fights lasted more than one day). Non-ant debris was
167 removed and remaining workers were dried and weighed (Mettler Electronic
168 Balance). The weight of 10 intact workers/10 gave an average individual worker
169 mass for each colony. Total casualties were estimated from the mass of tarp
170 casualties. Our casualty values are likely underestimated as wind removed some
171 dead ants from tarps in the field.

172 To parse winner and loser colony contribution to total casualties, we
173 genotyped (N=15-16) individuals collected from tarps of 5 of these 7 fight pairs
174 and matched them to their respective colonies following molecular protocols
175 (below). We calculated the cost to winner colonies as the total worker loss and
176 proportion of the initial colony lost.

177 *Vulnerability Associated with Fighting*

178 Newly acquired territory (as well as original host trees) may be
179 precariously defended by a diminished worker force after fights, and at risk from
180 attack by other space-limited neighbors. To assess vulnerability, we selected
181 single tree colonies similar in size to experimental fight trees as controls (N = 10
182 each for controls and experiment fights; Welch's t test for tree diameter
183 difference between groups $t_{13,0} = -1.74$, $P = 0.105$).

184 We examined changes in colony response to simulated large mammalian
185 herbivore browsing using methods modified from [34]. Two observers carefully
186 approached trees. Each visually identified an isolated branch with new growth,
187 and one swollen thorn domatia within 15 cm of the tip. Disposable 'mitts' crafted
188 prior to fieldwork (two sheets of paper towel 11"x6" folded over by 1 inch and

189 taped along 3 sides) were placed on each surveyor's right hand. With a leather-
190 gloved left hand, focal branches were simultaneously raked 3 times and then
191 enveloped by the mitt. Worker ants swarming on mitts after 30 seconds were
192 collected along with the mitt into a sealed bag. Bags were frozen and worker
193 contents subsequently counted. Surveys occurred on each of the 3 days before
194 experimental fights were induced (within tree replication $N = 6$; 2 surveyors x 3
195 days), and then again beginning 6 days after paired fights concluded.

196 Tree main stems are a primary access point for host invasion by ant
197 competitors [33,35], We used photographs taken 3 days before fights and again
198 6 days after to assess changes in colony defense of stems on the simulated
199 herbivory trees from above ($n=5$ photos from each period, 2 morning and 3
200 afternoon). Macro-digital photos always captured the south-facing plane of stems
201 and adjacent size standard ruler. We recorded the number of ant heads visible in
202 the frame from ground up to 10 cm.

203 Change in average response within colonies between controls and
204 experimental fights were analyzed using t -tests in JMP 8.0.

205 *Colony Relatedness and Enemy Adoption*

206 *Sampling / collection methods*

207 Individuals from $N = 18$ experimental colonies were analyzed using
208 molecular techniques. Before trees were tied together we collected pre-fight
209 workers from each experimental colony into a collecting vial (70% ethanol) in the
210 field.

211 For post-fight collections, we collected 5 domatia from each experimental
212 fight combined tree system on Sept 15, 2011 (hereafter 2mo) and March 22,

213 2012 (hereafter 8mo). Domatia were frozen, then opened and the contents
214 (mature and immature ants) were pooled into vials with 70% ethanol. For pre-
215 fight samples as well as 2mo samples, only intact adult workers were genotyped.
216 Individuals were inspected under a dissecting microscope to ensure they had no
217 appendages missing (indicating they were alive at the time of collection and not
218 cached casualties or emerged workers that were recently killed) and to exclude
219 body parts of other individuals. For 8mo samples we genotyped only immature
220 ants (nearly all worker pupae, but for colonies with < 24 pupae we extracted DNA
221 from large larvae or male pupae). Immature ants collected 8 months after fights
222 are unlikely to have been present at the time of conflict (Development time -
223 Appendix 1). Outcomes where all 8mo samples belong exclusively to one colony
224 suggest that the takeover was complete and resulted in the loser queen's death.
225 Alternatively, if genotypes matching both Pre-fight colonies could be found
226 among the immatures, an incomplete takeover is indicated (no loser can be
227 identified because both queens survived and continued to contribute to worker
228 production). Finally, novel genotypes that could not be explained by different
229 fathers but the same mother would suggest that a new queen was present within
230 the colony. This could occur through secondary takeover by non-relatives, or
231 possibly via the emergence of reproductive daughter queens [32]. For colonies in
232 each fight pair, sample sizes are listed in Table 1 but roughly follow: 8 workers
233 from each colony prior to fights (Pre-fight), then 48 workers and 24 immature
234 ants after 2 months and 8 months respectively.

235 *Lab protocols*

236 Individuals were extracted either using Qiagen DNA easy kits or Qiagen
237 Puregene extraction techniques. We genotyped Pre-fight and 8mo individuals
238 using 17 microsatellite loci (PCR protocols described in [36]).
239 For 2mo samples, where 48 individuals from each fight pair were analyzed, we
240 first determined which loci contained alleles that could distinguish between fight
241 pair colonies (from Pre-fight analysis results). We selected the 3-5 loci that
242 differed most in frequency between colonies, and included only primers from
243 those loci in the PCR reaction. If individuals could not be definitively assigned
244 with the subset of primers, they were re-run with more loci. PCR products were
245 run on a Capillary Electrophoresis Genetic Analyzer (an ABI Prism 3130) at
246 UCDNA Sequencing Facility at UC Davis and analyzed using GeneScan
247 software (Applied Biosystems, Carlsbad, CA, USA). Fragment data were
248 visualized and scored using STRand Version 2.3.69 [37].

249 *Analysis of genetic data*

250 Parentage for each worker was reconstructed using a maximum
251 likelihood approach implemented in COLONY v2.0.1.8 [38]. Null alleles and
252 scoring errors were accounted for using a 0.05 default error rate at all loci, and
253 no *a priori* relationships were assumed. Data for all individuals (from all fight pairs
254 at all times, $N = 765$) were combined for analysis. Individuals were separated into
255 full sibling or half sibling families, with associated probabilities of inclusion and
256 exclusion for each individual (Table 1). Queen number was also estimated, and
257 the identity of the maternal and paternal lineages at each sampling period were
258 reconstructed.

259 Allele frequencies obtained from COLONY analysis (above) were used to
260 calculate relatedness in COANCESTRY v 1.0.0.1 [39]. Relatedness values
261 between Pre-fight colonies were based on all genetic data from 17 loci. Because
262 2mo individuals were genotyped at a reduced number of loci, relatedness
263 estimates within individual winning colonies across all time points (Figure 2) were
264 based only on data from those restricted 3-5 diagnostic loci. For fight pairs where
265 genetic reconstruction did not reveal a distinct winner (ID3 and ID9), the Pre-fight
266 relatedness (Figure 2) used for statistical analysis is the mean of the among-
267 individual, within-colony relatedness for both Colony A and B. For each fight pair,
268 individuals were grouped by their sampling origin, Colony A Pre-fight, Colony B
269 Pre-fight, 2mo, 8mo, and compared to all individuals within the group to produce
270 average within-colony relatedness at each time period. We report Triadic
271 Maximum Likelihood estimators of relatedness coefficients, (TrioML) because
272 values are restricted to fall from 0-1, making interpretation intuitive. In contrast to
273 other pairwise relatedness estimators, this measure uses a third reference
274 individual to help minimize error [40]. To test for effects of fighting on relatedness
275 within colonies through time, we compared the TrioML relatedness values across
276 Pre-fight, 2mo, and 8mo sampling periods using standard least squares
277 regression, accounting for repeated measures by including Fight ID as a random
278 effect. Since TrioML relatedness coefficients are bounded at 0 and 1, we
279 performed arcsine square root transformations prior to analysis to better meet the
280 assumption of normality

281 RESULTS

282 *Costs of Fighting for Winners*

283 Estimated worker losses in fights between colonies ranged from 390 - 10
284 073 individuals ($N = 7$ colonies, $X + SE = 5,405 + 1,313$ individuals). A subset of
285 these casualties were genotyped and matched as full or half siblings to Colony A
286 or B pre-fight (Pre-fight) samples, all with a probability of assignment > 0.94 in
287 maximum likelihood sibship configurations from COLONY. Winning colonies
288 experienced high costs to fighting, with 19-56% ($X + SE = 39 + 7\%$, $N = 5$) of the
289 casualties collected for each fight pair belonging to the victors. Successful
290 colonies therefore lost on average 1/3 ($X + SE = 34 + 11\%$, $N = 5$) of their initial
291 worker force.

292 *Vulnerability*

293 Following fights, winner colony territories nearly doubled (proportion of
294 domatia pre- versus post-fight; $X + SE = 1.9 + 0.08$, $N = 14$) and defense of host
295 trees declined significantly. Canopy defense dropped by more than half
296 compared to Pre-fight levels as fewer ants from winner colonies responded to
297 simulated branch herbivory than from control colonies (t test: $t_{18} = -3.12$, $P =$
298 0.006 , Fig 1-A). Winner protection of stem access points also fell, with a
299 marginally significant difference between treatment and controls (t test : $t_{18} = -$
300 1.95 , $P = 0.067$ Figure 1-B).

301 *Colony Genetic Structure*

302 Pre-fight relatedness between colonies was low for all 9 fight pairs
303 analyzed using molecular markers (TrioML estimate of $r < 0.08$ for all pairs, Table
304 1). Sixteen of the 18 colonies were determined to contain full and half sib workers

305 produced by a single queen and two Pre-fight colonies were determined to
306 include workers produced by multiple queens (Table 1).

307 We infer three different outcomes from molecular analysis of fights;
308 complete rejection of non-kin, enemy adoption, and fusion. For fight pair ID's 7
309 and 4, all post-fight individuals ($N = 68 - 70$ from 2mo and 8mo) were assigned as
310 full siblings with individuals from only one Pre-fight colony (complete rejection of
311 non-kin). The remaining 7 pairs contained workers at 2 months after fights that
312 were matched as full siblings with individuals from both Pre-fight colonies
313 Consistent with enemy adoption, at 8mo, genotyped brood ($N = 23$ or 24) from 5
314 of these 7 pairs were assigned to only one of the Pre-fight colonies. For these
315 colonies, molecular data suggests a single queen-right colony succeeded in
316 conflict and subsequently included unrelated workers, but confirmed that loser
317 queen(s) were either no longer present or no longer contributing brood to the
318 colony at 8 months. Surprisingly, for fight ID's 3 and 9, genotyped brood at 8mo
319 were full siblings with individuals from both Pre-fight colonies, indicating that one
320 colony did not completely overtake the other and that both Pre-fight queens were
321 present and producing offspring. Furthermore, 16 individuals (76%) of 8mo
322 samples from fight ID 3 were classified by COLONY as full sibs with each other
323 (probability inclusion /exclusion >0.99) but were inferred to be the offspring of a
324 novel maternal genotype. Overall within-colony worker relatedness decreased
325 significantly from Pre-fight ($X + SE = 0.72 + 0.03$) to 2mo samples ($0.52 + 0.08$;
326 Linear mixed model: $R^2 = .80$, $P < .0001$; TukeyHSD for Pre-fight, 2mo
327 comparison: $P = 0.008$). Average relatedness for brood found within each colony

328 at 8 months after fights $X + SE = 0.68 + 0.08$) was similar to Pre-fight worker
329 relatedness (Tukey HSD for Pre-fight, 8mo comparison: $P = 0.760$) indicative of
330 restoration of pre-fight relatedness conditions at a colony level (Figure 2).

331

332 DISCUSSION

333 *Worker Losses and Vulnerability*

334 Are territorial fights between *C. mimosae* colonies costly to winners, and
335 does conflict weaken the victor's ability to defend resources? We found that
336 battle casualties reduce winner colony size by 1/3 on average, and as much as
337 2/3. In contrast to investigations highlighting advantages to combat victory
338 beyond resource acquisition (e.g. probability of success in future conflicts [33,35]
339 and better health [41]), we find that after successful fights, winning ant colonies
340 suffer from a window of vulnerability. *C. mimosae* winners are spread over twice
341 the territory (initial plus newly gained domatia) and appear susceptible to a loss
342 of territory value (e.g. removal of extrafloral nectaries, domatia, and reduced tree
343 growth) from mammal browsing and insect herbivory. Previous manipulations of
344 ant abundances on *A. drepanolobium* trees reveal a negative relationship
345 between colony size and branch damage by elephants and beetles [42,43]. The
346 actively growing shoot tips favored by large herbivores are also the site of
347 carbohydrate-rich extrafloral nectar production, which colonies rely on to fuel
348 activity and feed developing larvae [31,44]. Less than one week after
349 experimentally induced wars, colony defense of host tree canopies dropped by
350 66% compared to pre-fight levels (Figure 1). Simulated browsing of these
351 resources incited no defense on 3.5x the number of branches sampled after

352 fights as compared to before. Inability to protect tree-based energy sources
353 should hinder a colony's ability to produce and sustain workers. Additionally,
354 activity on tree trunks fell by 62% after fights, and we found a complete absence
355 of workers patrolling trunks in nearly 5x as many post-fight observation periods
356 as pre-fight observation periods. It is possible that nest-limited neighbors
357 discover the diminished resource holding capacity of winners either via territory
358 scouts [45] or by eavesdropping on the pungent alarm pheromones released
359 during combat [46,47]. Neighbors may then apply the information gained through
360 monitoring to target weakened competitors [48]. In a system where colony size
361 underlies competitive success [28], we document decreased worker number and
362 defense of hosts by winners. We hypothesize that public battles fought to gain
363 territory may subject victor colonies to increased risk of attacks and territory loss
364 [12,49].

365 *Non-kin Adoption During Recovery*

366 Are winner colonies built back through the adoption of losers? We found that
367 following fights, former non-kin enemies coexist within shared nests. In 56% of
368 the induced fights we analyzed genetically, despite prior lethal aggression
369 between competitors, post-fight colonies contained live workers that were full
370 siblings with the pre-fight loser colony (Table 1). In these cases, losers
371 represented an estimated 4-44% of post-conflict colonies' workforce. This
372 integration of losers in these cases was not consistent with queen-right (both
373 queens present) colony fusion because no brood developing within winner nests
374 matched loser genotypes at 8 months after fights. We conclude that for these five

375 colonies, loser queens were either killed or escaped during fights. We further
376 infer that their offspring (undeveloped brood, and possibly surviving workers) are
377 adopted by the victors, and act as an ephemeral resource for the winner colony.
378 Genetic similarity between winner and loser colonies does not explain variation in
379 the incorporation of non-colony members as all averaged pair-wise relatedness
380 values between fighting colonies were very low (relatedness coefficient $x < 0.08$
381 for all colony pair comparisons, $X + SE = 0.02 + 0.06$; 1st cousins should exhibit r
382 values of ~ 0.25 ; Table 1).

383 We estimate that 1,700-5,550 loser brood could remain within domatia
384 after experimental takeovers. This calculation is based on the average number of
385 brood per domatia ($X = 37.3$ from [50]) and the number of domatia on
386 experimental trees ($N = 48-150$). Since colonies in fighting pairs were estimated
387 to each contain a similar number of brood prior to conflict, adoption of all loser
388 brood would provide an instantaneous near doubling of new workers emerging
389 within the winner colony. Randomly selected *C. mimosae* pupae reared in the lab
390 become workers in an average of $X + SE = 8 + 0.74$ days, and well-developed
391 larvae pupate after an average of $X + SE = 12 + 0.72$ days (Appendix 1). Pupae
392 can metamorphose into fully formed workers with no tending or nutritional input
393 from adults (Appendix 1). Although complete worker development time (egg to
394 adult) is unavailable for this species, Argentine ants (*Linepithema humile*) display
395 pupal development rates that are similar to those observed for *C. mimosae* and
396 require an average of 63 days from oviposition to worker emergence [51].
397 Consequently, feeding loser brood to egg-laying queens or to larvae instead of

398 adopting them directly would create a months-long payoff lag. Retaining rather
399 than consuming loser brood may further boost colony size by stimulating the
400 surviving queen's egg production, as is seen in *Oecophylla* weaver ant colonies
401 that are experimentally augmented with non-kin pupae [52]. Adoption of
402 abundant non-kin brood is thus a more efficient way (in terms of both time and
403 energy) of converting loser individuals into valuable workers than a potential
404 alternative – cannibalism.

405 Sterile conspecific workers laboring for the fitness benefit of an unrelated
406 queen and/or colony - historically termed intraspecific slaves (but see [53]) –
407 have few documented examples in wild ant colonies [15,54]. This study
408 represents a rare example of this phenomenon, and the first evidence that non-
409 kin enemy adoption can be triggered experimentally in nature via conflicts
410 between large territorial colonies. Previous descriptions of natural non-kin
411 conspecific adoption/enslavement in ants come from species that are close
412 relatives to facultative and obligate interspecific slave-making taxa [15,55,56].
413 *Crematogaster*, a species-rich genus (476 known species - [57]), has no known
414 obligate slavemakers [15,21,58] and diverged from known obligate slave-making
415 species > 80 mya [59]. Like many other obligate plant ants and cavity nesters,
416 *Crematogaster* spp. are known to compete strongly with conspecifics for nest
417 space [60] and invade the domatia of heterospecific neighbors [35]. Conspecific
418 usurpation, though difficult to detect in nature, has been predicted [55] and
419 observed ([28], K. Rudolph pers. obs.). Our findings suggest that non-kin
420 adoption associated with territorial battles could be an overlooked phenomenon,

421 and potentially widespread in ants that engage in conflicts over nest sites or
422 foraging grounds.

423 *Additional Fight Outcomes*

424 Experimentally induced fights had multiple distinct outcomes. While five
425 winner colonies adopted non-kin orphans, two fights unexpectedly resulted in
426 queen-right colony fusions (Table 1). In fight ID's 3 and 9, genetic data indicates
427 that both Colony A and B queens were alive and producing brood within a shared
428 tree canopy 8 months after fights (Table 1). A unique feature of *C. mimosae*
429 seems to be that intense intraspecific aggression with extensive mortality can
430 rapidly give way to tolerance. In other documented cases of non-kin mergers,
431 worker interactions are rarely characterized by high aggression or lethality
432 [24,25,54,55,61–65]. Yet we find that in *C. mimosae* colonies mortal combat
433 transitions to coexistence over the course of hours (active fighting never lasted
434 for more than 48 hours, and most wars resolved in less than 12 hours). Not only
435 does aggression toward brood and callow workers cease, but it seems worker-
436 worker attacks do as well. It appears unlikely in the case of colony fusions that
437 queens could survive fights without some or many of their adult defenders also
438 persisting.

439 We do not yet know how de-escalation between fighting colonies may
440 proceed. It is possible that for conflicts between social insects generally, and
441 especially colonies with weak size asymmetry, assessment of fighting ability is
442 difficult [66]. Fights may escalate because the superior competitor cannot be
443 readily determined (failure of mutual assessment, as discussed in [49], or

444 because contested resources (e.g. nest space) are essential for colony survival
445 (i.e. there is no assessment; [67]). However, information indicating a growing cost
446 of conflict (e.g. fight duration) may induce de-escalation behavior in workers, and
447 protect against Pyrrhic victory. Reciprocal de-escalation in both colonies could
448 result in such a truce. Non-kin colony cooperation in times of vulnerability has
449 precedent in ants (e.g. joint colony founding ([22] and queenless colony fusion
450 [24]) but has been previously unreported in large, mature colonies as a result of
451 conflict. Regardless of the de-escalation mechanism, ultimate coexistence
452 appears impossible without strong temporal plasticity in templates of recognition
453 or chemical cues among adults.

454 Uncovering the patterns and mechanisms of recognition within and
455 between social insect colonies has long been an area of interest for biologists.
456 Two primary concerns are how and when the signals used in nestmate
457 discrimination are acquired. Chemical signals and perception underlying worker
458 exclusion or acceptance in a colony are not solely inherited; there can be
459 ecological and environmental effects on both [26,27,68]. Cooperation among
460 workers can be mediated by queen pheromones and/or learned based on
461 differences in cuticular hydrocarbons (CHC). These chemical signatures can be
462 modified by the environment and spread among individuals. In our cases of non-
463 kin adoption, winners' templates for tolerance appear to change as a result of
464 fighting. Non-kin losers may retain cues that winners detect as distinct but
465 overlook to enable acceptance of more individuals into the colony (active
466 adoption/ tolerance; [65]. Alternatively, through the extensive physical contact

467 involved in fighting, individuals may blend and dilute CHC signals, making non-
468 kin indistinguishable from one another (passive affiliation; [20,24,69]). We suggest
469 this system may be fertile ground for future studies examining how colony
470 chemistry is altered by conflict, especially as host tree takeovers are frequent.

471 Our third outcome showed that two winner colonies did not adopt non-kin
472 (at least in numbers appreciable in our samples, Table 1). During fights
473 representing each of our three outcomes discussed above, we occasionally
474 witnessed advancing workers ejecting brood from their opponent's domatia. If all
475 loser workers were eliminated in battle and this brood ejection behavior
476 continued, it could explain the lack of non-kin in all post fight samples for ID's 5
477 and 7. The findings of complete rejection, as well as temporary rejection followed
478 by acceptance of non-kin in this study, underscore the tension in colonies
479 between the benefits of incorporating non-kin versus the threat that non-kin could
480 represent to colony cohesion. Further exploration could help determine whether
481 reduced relatedness among nestmates following conflict has unexpected
482 consequences for colony function and the extent of recognition plasticity. For
483 example, do adoptees perform work within winner colonies and are some
484 colonies' signatures fundamentally incompatible with others?

485 *Conclusions*

486 Our findings represent a rare experimental quantification of the costs and
487 consequences of escalated fighting for winners in their natural environment.
488 Application of molecular analysis to a behavioral study exposed the leakiness of
489 colony boundaries in *C. mimosae*. Through field manipulations, we produced a

490 pattern long inferred by other researchers [17,55,56,61,70] that ant conflicts over
491 territory predictably decrease within-colony relatedness (via non-kin enemy
492 adoption and colony fusion Fig 2), and that colony cohesion appears robust to
493 this perturbation. Importantly, this non-kin affiliation occurs within large mature
494 colonies, not recently founded ones [71] A study of conflict in wood ants showed
495 that violent wars fought to expand territory produced casualties that were fed to
496 developing larvae [72]. Our work points to a different, potentially
497 underappreciated source of profit for colonies that succeed in conflict. After costly
498 contests, *C. mimosae* winners at times gain not only valuable new host trees but
499 also living spoils of war in the form of non-kin adoptees that provide victors with
500 an accelerated means to colony size recovery.

501

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512

513

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- 717
718
719

1 Table 1.

2 Sampling design, colony relationships, and experimental fight outcomes for *Crematogaster*
3 *mimosae*

4

5 Figure 1

6 Vulnerability following conspecific conflict. Winner colonies decreased defense after battles.

7 Mean +SE change in the number of ants guarding host trees in canopies (A) and on main stems
8 (B) relative to pre-fight levels. Unmanipulated controls did not show a decrease in defense over
9 the same period.

10

11 Figure 2

12 Average within colony relatedness over time for each fight manipulation. Pre-fight relatedness
13 values are from fight winner colonies (except for ID3 and ID9). For these two colonies where no
14 definitive loser could be identified, values are an average of within Pre-fight Colony A and B
15 relatedness. Workers collected 2 months after fights were significantly less related on average
16 to their nestmate contemporaries than workers sampled before fights and brood sampled 8
17 months after ($P < 0.0001$). Winner colonies ID4 and ID7 (open symbols) contained no loser
18 genotypes 2mo after fights. Solid symbols identify colonies with enemy adoptees (loser
19 genotypes present at 2mo but not at 8mo). Line symbols identify colonies with no definitive fight
20 winner where colony fusion is inferred (both pre-fight colony genotypes are present in workers
21 at 2mo and brood at 8mo).

22

23 Figure 3

24 Images of experimental fights. Conflict induced by joining host tree canopies of separate
25 colonies (A) with a tarp to catch casualties. (B) *Crematogaster mimosae* battle in progress on
26 the surface of a contested domatia.

27 Table 1..

Fight pair ID	sample n (A, B, 2mo, 8mo) ^a	# loci (A, B, 2mo, 8mo) ^b	# queens (A, B, 2mo, 8mo)	Winner (behav assay)	Winner (molecular data)	Adopt enemies ^c	Prop adoptees (at 2mo)	Between colony relatedness (A vs B) ^d	A and B genotypes present after 8mo? ^e
4	8, 8, 48, 22	17, 17, 4, 17	1,1,1,1	unclear	A	no	0	0	no
7	8, 8, 44, 24	17, 17, 5, 17	1,1,1,1	unclear	B	no	0	0	no
1	8, 8, 47, 24	17, 17, 4, 17	1,1,2,1	unclear	B	yes	0.21	0	no
2	8, 8, 46, 23	17, 17, 4, 17	1,2,3,1	A	A	yes ★	0.17	0.08	no
5	8, 8, 47, 23	17, 17, 4, 17	1,1,2,1	A	A	yes	0.44	0.04	no
6	8, 8, 47, 23	17, 17, 3, 17	1,1,2,1	A	A	yes	0.04	0	no
8	8, 8, 45, 24	17, 17, 4, 17	1,1,2,1	B	B	yes	0.04	0	no
9	8, 8, 47, 23	17, 17, 5, 17	1,2,3,2	unclear	?	°	fusion	0.04	yes
3	8, 8, 46, 21	17, 17, 4, 17	1,1,2,3	A	?	unclear #	fusion	0.03	yes

28

A = individuals collected from A tree (Pre-fight)

B = individuals collected from B tree (Pre-fight)

2mo = individuals from joined A+B trees (2 months after fight)

8mo = individuals from joined A+B trees (8 months after fight)

^a n - number of individuals (indv) genotyped from each period (A, B, 2mo all indv = workers; 8mo indv = pupae or larvae)

^b 2mo loci restricted to those with diagnostic alleles differentiating A and B genotypes

^c Proportion of sample individuals assigned as full sibs with loser colony genotype with (inclusion/exclusion probability > 0.99).

Exceptions-

★ ID 2 - half sibs with B = 7 indv ($p > 0.95$)

° ID 9 - full or half sib with B = 20 indv ($p > 0.85$), full sib with A = 28 indv ($p > 0.99$)

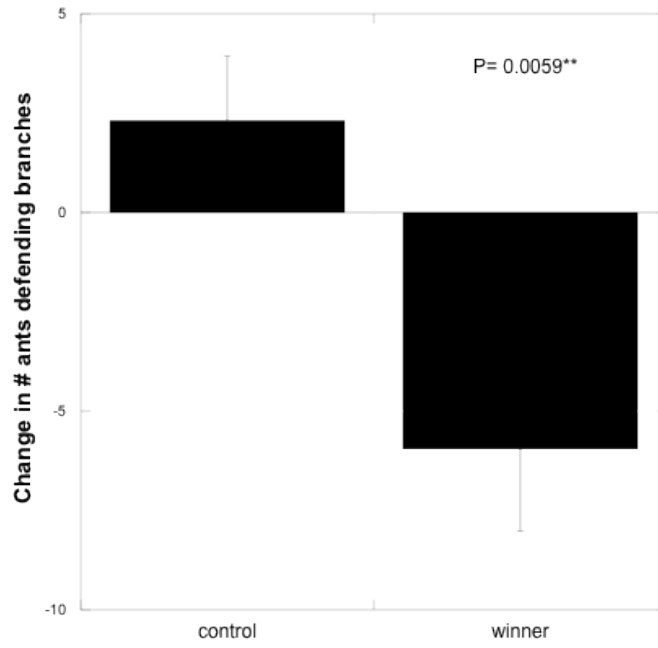
ID 3 - full sib with B = 24 indv, A = 22 indv ($p > 0.99$)

^d average pairwise relatedness (TrioML estimator in COANCESTRY (Wang 2011))

^e no = all brood excluded as full sibs with loser genotype at $p > 0.99$; for yes – ID 9 (12 indv full sib with A, 11 indv with B),

ID 3 (4 indv full sib with A, 1 indv with B, 16 indv were offspring of a new queen).

A



B

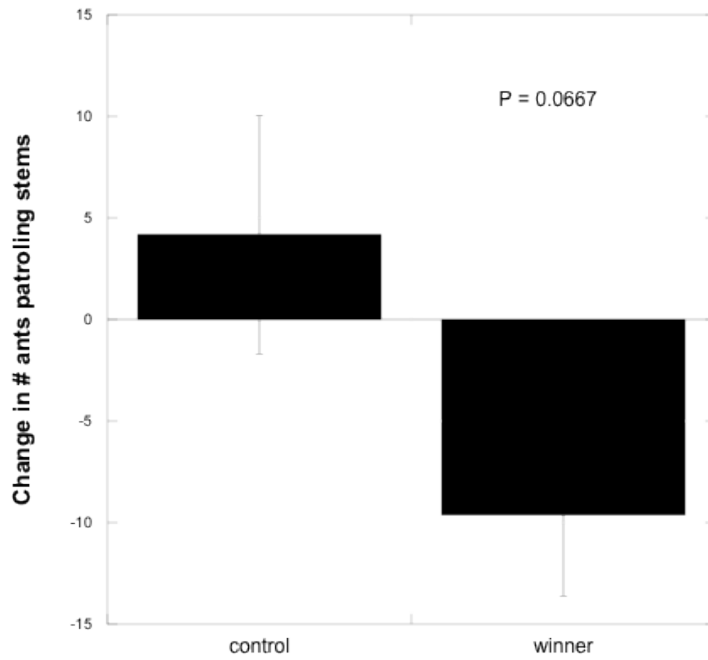


Figure 1.

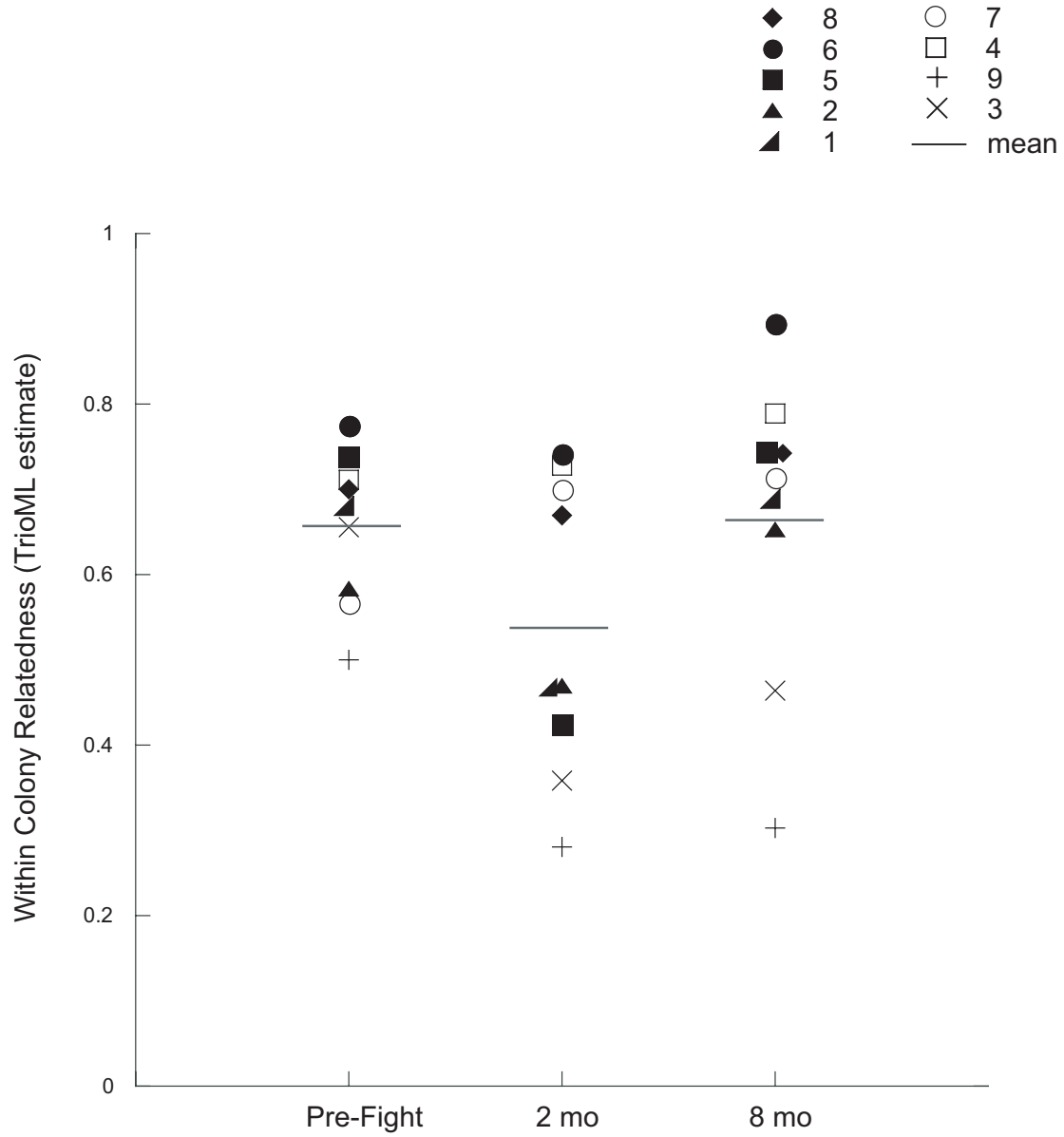


Figure 2.



A



B

Figure 3. *(in color web and print)*