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COMPARING THE COSTS AND BENEFITS OF GROUPING WITH
NON-RELATIVES IN THE SOCIAL AMOEBA *Dictyostelium discoideum*
(AMOEBAZOA) AND THE SOCIAL WASP *Polistes dominulus*
(HYMENOPTERA VESPIDAE)

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Strassmann J., Queller D., Emerson J., Stagi M., Cervo R., Turillazzi S. – Cooperation and conflict among non-relatives in the social amoeba *Dictyostelium discoideum* (Amoebazoa) and the social wasps *Polistes dominulus* (Hymenoptera Vespidae)

Theory of social organization based on Hamilton's rule and the importance of genetic relatedness should hold not only for social insects, but also for social micro-organisms. Here we compare individual behavior in social groups that contain both relatives and non-relatives. We find that in wasps groups containing non-relatives are not markedly different from those with relatives in nest growth. By contrast there are real differences in social amoebae when groups contain non-relatives. Slugs that are chimeric migrate less far than those that are pure clones. Some clones contribute more to the sterile stalk cells than their partners in chimeras, a kind of cheating. Grouping with non-relatives is common in both species, most likely because of the great advantages of large groups. It is likely that the social amoebae recognize non-relatives while the wasps either do not recognize non-relatives, or do not treat them differently because this would cost the group too much.

KEY WORDS: kin selection, unrelated groups, *Polistes dominulus*, *Dictyostelium discoideum*, sociality.

INTRODUCTION

Hamilton revolutionized the study of social organization with his insight that genes could be transmitted by helping relatives other than progeny (HAMILTON, 1964). His rule for when kin should be aided is $rB - C > 0$, where r is genetic relatedness between the altruist and the beneficiary, B is the number of extra progeny raised by the beneficiary because of the assistance of the altruist, and C is the cost to the altruist in the form of forgone reproduction. This insight started the field of sociobiology and behavioral ecology, and has proven to be very powerful in explaining the occurrence of altruism (WILSON, 1975). But this does not mean that all groups contain only relatives. Groups can consist of non-relatives if individuals obtain direct benefits from grouping (ALEXANDER, 1974). Groups containing non-relatives who behave altruistically towards others can also occur under specific circumstances. These include cases where there are errors in recognition, cases where excluding non-relatives is not worth the cost of doing so, and cases where there is continuing conflict over who ultimately benefits from the group (BERNASCONI & STRASSMANN, 1999).

In this study we compare groups with non-relatives to groups with only relatives in two very different organisms, the social amoeba, or cellular slime mold, *Dictyostelium discoideum* and the social wasp *Polistes dominulus*. Social amoebae are soil dwelling predatory protists that eat bacteria and divide by mitotic fission as long as there is plenty of food in the environment (RAPER, 1984). When they begin to starve, they aggregate to a cAMP signal and form a motile slug (KESSIN, 2001). This slug moves towards heat and light and away from ammonia, and ultimately forms a fruiting body consisting of a sterile stalk and spores. About 20% of initially independent cells die to form the stalk, while the others form the spores. Since the social stage is the result of aggregation,

unrelated individuals can join the same fruiting body, setting up the possibility of conflict, exploitation, and diminished function (QUELLER *et al.*, 2003; STRASSMANN *et al.*, 2000).

Polistes dominulus foundresses begin nests together in Spring. All are mated and are capable of being independent queens, yet they often form groups in which most give up reproduction and one becomes the functional queen, though another will take her place if she dies. *P. dominulus* foundresses aggregating to form groups in which some help others reproduce can be compared to *D. discoideum* amoebae aggregating to form groups in which some help others disperse as spores. Here we look at the role of relatedness in these processes, and whether competition and exploitation is greater in groups containing non-relatives than it is in groups of only relatives.

MATERIALS AND METHODS

We observed chimeric clones of *D. discoideum* that had been collected in nature from Mountain Lake Virginia and Little Butt's Gap, North Carolina (STRASSMANN *et al.*, 2000; FOSTER *et al.*, 2002). Chimera formation followed standard protocols using equal numbers of cells of each of two, five or ten clones (www.ruf.rice.edu/~evolve; FOSTER *et al.*, 2002). We initially held total cell number constant, then compared different cell numbers. We knew the different clones were genetically distinct because we genotyped them at several microsatellite loci, and they had distinct alleles (STRASSMANN *et al.*, 2000). We looked at function of chimeric groups in two ways. We examined whether the chimeric group as a whole migrated as effectively as did a group of clonemates. We also looked at whether contribution to fertile spore tissue was equal between two competing clones in the same chimera.

We censused and collected *Polistes dominulus* foundresses in

a 3 ha section of a reforestation project near Cavriglia in Arezzo Province of Tuscany. *P. dominulus* choose to nest in open ended plastic tubes that protect saplings in the reforestation area. Overwintered foundresses initiate colonies singly or in groups in early spring, usually in early to mid March. We studied 62 nests from 18 March until 17 April 1997.

We censused the nesting sites early in the morning before the wasps became active. We individually marked wasps tending newly initiated nests in the sapling tubes. We also marked newly arrived foundresses in our daily censuses as they joined nests throughout the observational period. Before any larvae pupated, at the conclusion of the census period on 17 April, we collected the 44 surviving nests and their associated foundresses (n=140) and stored them at -80°C for later genetic analysis.

We genotyped these samples for five trinucleotide microsatellite loci designed for *P. dominulus*: Pdom122AAT, Pdom 140TAG, Pdom20CAT, Pdom20AAG, and Pdom7AAG (HENSHAW, 2000). We followed standard methods for DNA extraction, PCR amplification, and resolution of PCR (STRASSMANN *et al.*, 1996).

We estimated relatedness using Relatedness 5.05 (GOODNIGHT & QUELLER, 1999). We sorted individual foundresses into full-sibling groups using a likelihood method implemented by the computer program Kinship 1.3 (GOODNIGHT & QUELLER, 1999). In our study, we calculated the ratio of likelihoods of a pair of individuals having a haplodiploid full sister relationship over the null hypothesis of no relatedness. We established the significance of the ratios by simulating genotypes for 1,000 random pairs of sisters and 1,000 random pairs of unrelated individuals using the observed allele frequencies in the population as a whole. Kinship sorted the $N \times N$ half matrix of likelihood ratios according to an algorithm that clusters likely relatives near one another. We then inspected these putative full sibling groups and assigned likely maternal and paternal genotypes based on the other members of the group. We excluded those members that did not match the consensus parental genotypes. When individuals lacked significant likelihood ratios, we still included them if they matched the parental genotypes.

Foundresses frequently visited other nests during the census period. We designated foundresses as *movers* if the nest of their marking differed from the nest on which we collected them. Though we detected a large amount of short term visits, relatively few foundresses actually moved permanently from their nests of origin to the nest on which we collected them. For the 21 wasps that did move, we tested the roles of factors that might condition their decision to move. These factors include nest size, nest productivity, relatedness, wasp size, and reproductive capacity of nestmates.

We measured the ovaries of 140 foundresses as an indicator of reproductive status. For each foundress with an intact abdomen, we counted the number of mature and nearly mature eggs and measured the length of the largest egg or oocyte. We measured the length of the longest cell in the wing as a size measure. We standardized these size measures so that they could be compared among colonies by calculating the deviation of the size from the mean size of the nest and dividing by mean size of the nest.

RESULTS

Chimeras of *D. discoideum* did not migrate as far as did pure clones when total cell number was held constant which indicates that there is a cost to chimerism (FOSTER *et al.*, 2002). If just cell number is varied, slugs with more cells moved farther. When the two factors were varied together, we found that the advantage of larger group size outweighed the

cost of chimerism in migration distance (FOSTER *et al.*, 2002). In chimeras one clone exploited the other by failing to contribute its fair share to making the sterile stalk in 6 of 12 cases (STRASSMANN *et al.*, 2000).

In *Polistes dominulus* on average, 3.2 ± 1.8 ($\bar{x} \pm s.d.$) foundresses tend each of the 44 nests, ranging from one to eight. The average sibship, numbers of full sisters in the population (N=83) regardless of what nest they were on, was 3.5 ± 1.5 ($\bar{x} \pm s.d.$) sisters ranging from two to six. Fifty-seven females had no sisters collected by us, though they might have had sisters on other, uncollected nests.

The overall relatedness among the 36 multiple foundress nests of the entire population was 0.12 ± 0.027 , an extremely low value for relatedness among foundress associations in *Polistes* species, even lower than the 0.36 value from the same year in a nearby population (figure I; QUELLER *et al.*, 2000). Of the 36 nests collected with multiple foundresses, 24 showed relatednesses consistent with little or no relation ($R < 0.2$) while only 7 nests showed a higher R consistent with a mixture of relatives and non relatives ($0.2 < R < 0.6$). The remaining 5 nests had R values consistent with nearly full sister nests ($R > 0.6$).

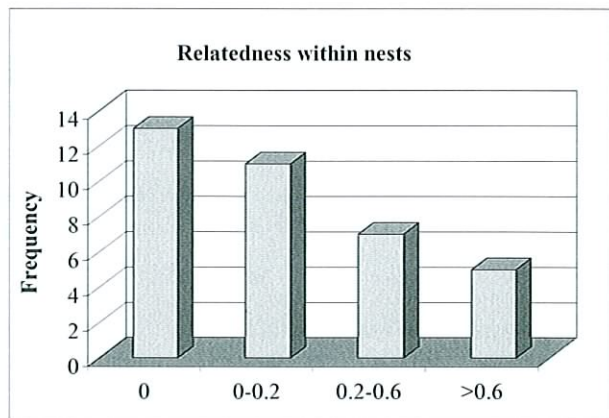


Figure I – Genetic relatedness among foundresses on the same nest of *Polistes dominulus*. Values of zero or below are indicated as zero.

Foundress associations in the study population show the expected cooperation among members, as the number of nest cells per colony increased with increasing foundress number (regression slope=7.3, $R^2=0.552$, $P < 0.0001$, figure II). However, increasing relatedness does not lead to a corresponding increase in the number of cells per foundress (regression slope=2.374, $R^2=0.036$, $P=0.27$, figure III) or total number of cells per colony (regression slope=13.53, $R^2=0.061$, $P=0.15$), indicating that colony mates cooperate independently of relatedness. In addition, average relatedness within colonies remains independent of association size. Larger associations do not lead to lower relatednesses (regression slope=0.027, $R^2=0.019$, $P=0.42$). Thus, larger colonies in the population do not unduly inflate the low relatedness calculated for this population.

Of the twenty-one wasps that switched nests, ten moved to nests with smaller members, ten to nests with larger members and one moved to a nest of similarly sized females. Clearly, movers did not increase their relative body size when moving from one nest to another (Wilcoxon signed rank test $P=0.60$, paired sign test $P > 0.99$, paired t-test $P=0.40$, $n=21$). Females do not move to a new nest where they could be more dominant because they are larger.

Foundresses did not move to nests where their ovaries were more developed than average for number of mature eggs, nearly mature eggs, or size of largest oocyte (Wilcoxon signed rank test $P=0.48$, paired sign test $P > 0.99$, paired t-test $P=0.45$

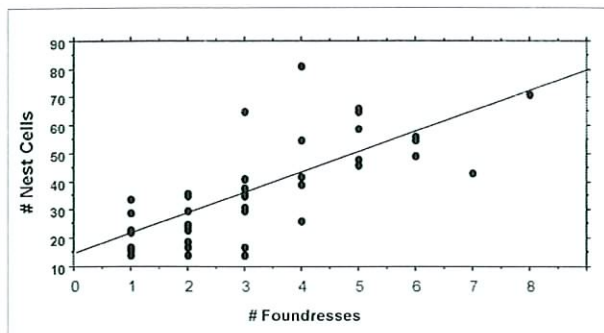


Figure II – Colonies with more foundresses build more cells (regression slope=7.3, $R^2=0.552$, $P<0.0001$).

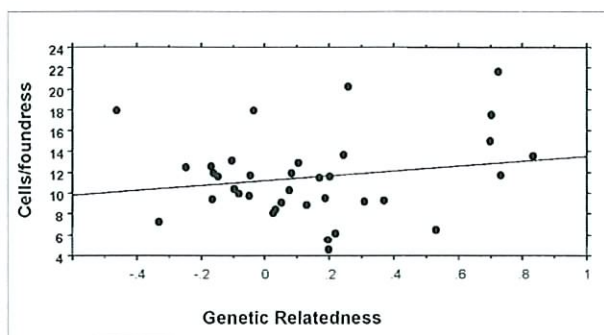


Figure III – Colonies with higher relatedness do not have a higher number of cells constructed per foundress (regression slope=2.374, $R^2=0.036$, $P=0.27$).

for mean number of mature eggs, $n=21$; Wilcoxon signed rank test $P=0.41$, paired sign test $P=0.33$, paired t-test $P=0.36$, $n=21$ for mean number of nearly mature eggs; Wilcoxon signed rank test $P=0.59$, paired sign test $P>0.99$, paired t-test $P=0.41$, $n=21$ for mean size of largest oocyte).

However, wasps may have moved to nests with more foundresses than their original nests, suggesting advantages to larger association sizes. Of the twenty one movers, fifteen wasps moved to nests with a greater total number of foundresses even though the difference is not statistically significant ($P=0.078$, two-tailed sign test).

DISCUSSION

Both the social insect and the social amoeba studied here form groups consisting of relatives and non-relatives. They differ in that the social amoeba groups are mainly comprised of clonemates and non-relatives while the social wasps have non-relatives, full sisters, and more distant relatives (FORTUNATO *et al.*, 2003; QUELLER *et al.*, 2000). Both species join with non-relatives into a cooperative endeavor in attempts to increase group size. In both species the role of non-reproductive helping is unevenly divided among kin groups; in *D. discoideum* some clones contribute more to the sterile stalk cells than do their partners in a chimera. In *P. dominulus* one queen monopolizes most reproduction and the other foundresses serve as her largely sterile helpers. Whether joining non-relatives actually benefits the joiners is difficult to say in each species.

In *P. dominulus*, females will leave smaller groups to join larger ones irrespective of relatedness. Foundresses of *P. carolina*, which nest exclusively with full sisters, will also leave smaller groups to congregate on larger nests, so that ultimately no single-foundress nests remain (SEPPÄ *et al.*, 2002). This

indicates there must be a large advantage to groups, greater than the advantage in cell number measured at the end of the foundress period. Like *P. dominulus*, *P. carolina* nests in cavities. Larger group size may be critical in defending the cavity from other wasps, or other cavity nesting species.

Our study of *P. dominulus* was a natural one conducted in the field, so specific field conditions could influence the results. The colonies of our population were built in open ended plastic tubes which do not give protection against bad weather. After the winter no combs were found in our nesting field. The lack of old combs probably does not favour foundresses return to the natal nest before spring foundation and the association among relatives. At more established nest sites relatedness could be much more important, with females from the same nest of the previous year beginning a new nest with sisters the subsequent year.

Since there is no indication that relatedness affects foundress behavior in *P. dominulus*, one possibility is that they cannot distinguish relatedness. However, some of us have recently demonstrated that the epicuticular lipids vary with relatedness in *P. dominulus* though it is not yet clear whether or how wasps use this information (DANI *et al.*, 2004). Moreover, D'APPORTO *et al.* (2004) report that cuticular hydrocarbons blends of the overwintering females can change when they hibernate in clusters which affects recognition among relatives in the spring associations. Therefore, returning to the natal nest to find relatives could be less error-prone than straight chemical recognition.

If the females cannot recognize relatives, strategies would be based on the expected relatedness dependent on nest site, or other cues. Subordinates would also obtain some indirect benefits by, on average, helping kin. The expected benefit of helping may be somewhat higher than the 0.12 relatedness would suggest, since relatedness is more often around 0.3 in neighboring populations (QUELLER *et al.*, 2000).

Our study of *D. discoideum* was done under laboratory conditions and so therefore indicates the potential of what may happen in the field. The laboratory results are likely to be robust for the finding that amoebae group with non-relatives, that such groups are less effective at migrating, and that larger groups migrate farther than smaller groups. What we cannot say from this study is how often both these costs and benefits arise in nature. It is likely that other individual amoebae are even more limiting in nature than in the laboratory, increasing the benefit of grouping. It is also likely that non-relatives are encountered since we found genetic diversity in clones at the scale of 0.2 gram samples (FORTUNATO *et al.*, 2003). Future work is needed to see how common this is in the wild, and how great is the advantage of migrating farther.

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RIASSUNTO

CONFRONTO DEI COSTI E DEI BENEFICI IN AGGREGAZIONI DI NON PARENTI NELL' AMEBA SOCIALE DICTYOSTELIUM DISCOIDEUM E NELLA VESPA SOCIALE POLISTES DOMINULUS

La teoria dell'organizzazione sociale basata sulla regola di Hamilton e l'importanza della parentela genetica dovrebbe valere non solo per gli insetti sociali, ma anche per i

microorganismi sociali. In questo lavoro, confrontiamo il comportamento individuale in gruppi sociali che contengono sia parenti che non parenti. Emerge che la crescita delle colonie composte da gruppi di vespe non parenti non è sostanzialmente differente da quella delle colonie composte da parenti. Al contrario, esistono reali differenze nelle amebe sociali quando i gruppi contengono non parenti. Le «chimere» percorrono distanze minori durante le migrazioni rispetto ai gruppi costituiti da cloni puri. Alcuni cloni contribuiscono più al gambo sterile di quanto non fanno i loro partners delle chimere, una sorta di imbroglio. Il raggrupparsi con non parenti è comune in entrambe le specie, probabilmente per il vantaggio dato dai gruppi numerosi. E' probabile che le amebe sociali riconoscano non parenti mentre le vespe non siano capaci né di riconoscere i non parenti, né di trattarli in modo differenziale perchè questo costerebbe troppo al gruppo.

REFERENCES

- ALEXANDER R.D., 1974 - *The evolution of social behavior*. - Annu. Rev. Ecol. Syst., 4: 25-383.
- BERNASCONI G., STRASSMANN J.E., 1999 - *Cooperation among unrelated individuals: the ant foundress case*. - Trends Ecol. Evol., 14: 477-482.
- DANI F.R., FOSTER K.R., ZACCHI F., SEPPÄ P., MASSOLO A., CARELLI A., AREVALO E., QUELLER D.C., STRASSMANN J.E., TURILLAZZI S., 2004 - *Can cuticular lipids provide sufficient information for within-colony nepotism in wasps?* - Proc. R. Soc. Lond. B, 271: 745-753.
- DAPPORTO L., PANSOLLI C., TURILLAZZI S., 2004 - *Hibernation clustering and its consequences for associative nest foundation in Polistes dominulus (Hymenoptera, Vespidae)*. - Behav. Ecol. and Sociobiol., 56: 315-321.
- FORTUNATO A., STRASSMANN J.E., SANTORELLI L.A., QUELLER D.C., 2003 - *Co-occurrence in nature of different clones of the social amoeba, Dictyostelium discoideum*. - Mol. Ecol., 12: 1031-1038.
- FOSTER K.R., FORTUNATO A., STRASSMANN J.E., QUELLER D.C., 2002 - *The costs and benefits of being a chimera*. - Proc. R. Soc. Lond. B, 269: 2357-2362.
- GOODNIGHT K.F., QUELLER D.C., 1999 - *Computer software for performing likelihood tests of pedigree relationship using genetic markers*. - Mol. Ecol., 2: 1231-1234.
- HAMILTON W.D., 1964 - *The genetical evolution of social behaviour: I-II*. - J. Theor. Biol., 7:1-52.
- HENSHAW M., 2000 - *Microsatellite loci for the social wasp Polistes dominulus and their application in other polistine wasps*. - Mol. Ecol., 9: 2155-2157.
- KESSIN R.H., 2001 - *Dictyostelium: evolution, cell biology, and the development of multicellularity*. Cambridge University Press, Cambridge, pp. 394.
- QUELLER D.C., FOSTER K.R., FORTUNATO A., STRASSMANN J.E., 2003 - *Cooperation and conflict in the social amoeba, Dictyostelium discoideum*. In: Genes, Behaviors, and Evolution of Social Insects, Kikuchi T., Kubo T. & Higashi S. (Eds.) Hokkaido University Press, Sapporo, Japan, pp. 173-200.
- QUELLER D.C., ZACCHI F., CERVO R., TURILLAZZI S., HENSHAW M.T., SANTORELLI L.A., STRASSMANN J.E., 2000 - *Unrelated helpers in a social insect*. - Nature, 405: 784-787.
- RAPER K.B., 1984 - *The Dictyostelids*. - Princeton University Press, Princeton NJ, pp. 453.
- SEPPÄ P., QUELLER D.C., STRASSMANN J.E., 2002 - *Reproduction in foundress associations of the social wasp, Polistes carolina*. - Behav. Ecol., 13: 531-542.
- STRASSMANN J.E., SOLÍS C.R., PETERS J.M., QUELLER D.C., 1996 - *Strategies for finding and using highly polymorphic DNA microsatellite loci for studies of genetic relatedness and pedigrees*. In: Molecular Zoology: Advances, Strategies and Protocols, Ferraris J. D. and Palumbi S. R., Eds., Wiley-Liss, Inc., New York, NY, Pp. 163-180, 528-549.
- STRASSMANN J.E., ZHU Y., QUELLER D.C., 2000 - *Altruism and social cheating in the social amoeba, Dictyostelium discoideum*. - Nature, 408: 965-967.
- WILSON E.O., 1975 - *Sociobiology: The new synthesis*. - Cambridge MA, pp. 697.