

Analysis of Two Genetic Models for the Innate Components of Colony Odor in Social Hymenoptera

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Summary. We propose two models for the inheritance of the innate components of colony odor in social Hymenoptera. Under the Individualistic model, individuals are hostile unless they share at least one allele at all colony-odor loci. Under the Gestalt model, colony-odor pheromones are transferred between individuals, resulting in a 'gestalt' colony odor; colonies will not fuse unless they have the same genetic mix of workers. We analyze these models for the case of colonies founded by single, once-mated queens. The Gestalt model seems generally favored for most species, although some evidence suggests the Individualistic model may operate in some primitive ants. A single colony-odor locus is possible in the Gestalt model, but seems improbable in the Individualistic case. We propose a general inbreeding test to estimate the number of loci involved in colony odor.

Introduction

The essence of social insects is that they live in colonies. With few exceptions, it is also true that these colonies resist mixing with other colonies, even of the same species. Individuals recognize those of other colonies through differences in 'colony odor.' The source of colony odor has been much discussed and also experimentally sought after, but without, to our minds, any satisfyingly conclusive consensus having been reached. In this paper we examine one aspect of this problem as it applies to social Hymenoptera.

There are two possible sources for colony odor: from the insects themselves (with genetic variation being the basis for odor differences between colonies), and from the food and surroundings of the colony (with environmental variation being the basis for odor differences between colonies). In a significant review of the earlier literature, Ribbands (1965) concluded that colony odor in ants is primarily genetically determined, but that in honeybees it is environmentally determined. In fact, most recent investigators seem to favor one or other mechanism as being the only one operating, whereas we suggest that social Hyme-

noptera can use either environmentally derived odorants, from food or via absorption of local odorants into the cuticular waxes, or innate, genetically determined pheromones which in some cases may be reinforced by environmental components.

The fact that environmental influences on colony odor can and have been repeatedly and readily demonstrated has led to attempts to exclude any genetic component. Such argumentation has been inconclusive. Thus, colony-odor differences in *Apis mellifera* were readily accentuated by differential feeding (Ribbands, 1965), but the splitting of colonies in the attempt to eliminate genetic variation ignores the unusually large amount of variation in honeybee colonies due to multiple insemination of the queen (resulting in the likelihood that the two halves differ in their genetic mixes); such halves develop hostility even when fed the same food (Chauvin, 1968, p. 66). Various recent experiments on ants (Hangartner et al., 1970; Hubbard, 1974) and, especially, on bees (Barrows et al., 1975) suggest that there is a genetic component to colony odor in at least some social Hymenoptera, but confirmation of these results is needed, along with their extension to other species. We now propose two simple models for the inheritance of this odor, and examine population genetic characteristics of these models.

The Models

We propose that innate colony-odor differences between the colonies of social Hymenoptera are determined by allelic variation at a number of colony-odor loci. Workers 'recognize' each other as nestmates if they share at least one allele for each odor locus. Our hypotheses thus differ from that of Brown (1968), who suggests that colony odors function by marking strangers rather than by indicating nestmates.

Our 'Gestalt' model supposes that colony-odor pheromones are transferred by grooming or trophallaxis among all the individuals of the colony, so that workers respond to a common gestalt odor, rather than to that which they each produce individually.

Our 'Individualistic' model supposes that each colony member retains its pheromonal integrity, with no significant transfer of colony-odor pheromones between colony members.

Analysis of the Models

We now ask how much genetic variation is necessary at colony-odor loci under our models to yield the levels of intercolony distinctness commonly observed in natural populations. We analyze one particular type of colony organization thought to be common among social Hymenoptera: that where the colony consists of a single, once-mated queen and her worker progeny (see Crozier, 1977, for a discussion of the analysis of colony structure in social Hymenoptera), remembering that hymenopterans males are haploid. We assume that intercolony distinctness is advantageous and hence alleles promoting this distinctness are

selected for. It follows from this assumption that, as is the case with self-incompatibility alleles in plants (Wright, 1969, pp. 402–406) and sex-determination alleles in Hymenoptera (Crozier, 1971), alleles at a colony-odor locus will be selected for when rare, against when common, and tend to reach equal frequencies. The final simplifying assumptions are that each colony-odor locus has the same number of alleles, that these have reached the same or similar frequencies, that the distortion of genotypic frequencies from those expected under the Hardy-Weinberg law is small, and that the queen's contribution to the colony odor of mature colonies is negligible.

We now derive expressions giving the probability, P , that any one colony will remain distinct from each of x neighbors because of the action of z colony-odor loci each with n alleles.

1. General Quantities

By definition, the frequency, q , of an allele at a colony-odor locus is:

$$q = 1/n. \quad (1)$$

At any one locus, the total frequency of homozygous queens will be:

$$H_o = n \cdot q^2. \quad (2)$$

At any one locus, the total frequency of heterozygous queens will be, from (1) and (2):

$$H_e = 1 - q = (n - 1)/n. \quad (3)$$

2. Gestalt Model

Under the Gestalt model, colonies will be pheromonally completely distinct unless they have the same worker genotype mix.

Colonies founded by homozygous queens can be divided into a proportion q that have mated with males carrying an allele identical to their own, and a proportion $1 - q$ that have mated with a male of a different haploid genotype. Those that have mated with a male carrying the same allele as theirs will yield colonies that only fuse with others resulting from exactly the same parental genotypes, so that, for one locus, the probability of such a colony fusing with a single neighbor is:

$$H_{1os} = q^3. \quad (4)$$

Colonies resulting from a homozygous queen mated with a male of different genotype will fuse with colonies resulting from the same parental genotypes, or the complementary genotype mix, because both give rise to the same set of heterozygous workers (e.g., $AA \times B$, and $BB \times A$), so that the chance of fusion with a neighbor is in this case:

$$H_{1od} = 2q^3. \quad (5)$$

For heterozygous queens, fusion of the resulting colonies is only possible between colonies with identical parental genotypes, so that for a queen mated to a male carrying an allele identical to one of hers (a proportion $2q$ of all heterozygous queens) the probability of fusion with a neighbor is:

$$H_{1es} = 2q^3 \quad (6)$$

and the probability for a queen mated to a male carrying a third allele (e.g., AB \times C) is:

$$H_{1ed} = 2q^3. \quad (7)$$

Weighting each colony type by its frequency, from (1), (2), (3), (4), (5), (6), and (7), the proportion of the population of colonies capable of fusion with a given neighbor is:

$$M_1 = q^3 (2 - q^2). \quad (8)$$

Hence, the probability of any two colonies being incapable of fusion, given z loci, is:

$$P_{11} = 1 - M_1^z \quad (9)$$

and the probability of a randomly chosen colony being incapable of fusion with any of x neighbors is:

$$P_{1x} = (1 - M_1^z)^x. \quad (10)$$

3. Individualistic Model: Fusion

By inspection of the array of colonies and determining which types are capable of fusion, through having all workers in each compatible with one another, we obtain for this model similar quantities to those derived for the Gestalt model:

$$H_{2os} = q + q^2 - q^3 \quad (11)$$

$$H_{2od} = 2q + 4q^2 - 8q^3 \quad (12)$$

$$H_{2es} = q + q^2 - q^3 \quad (13)$$

$$H_{2ed} = q + q^2 + 5q^3. \quad (14)$$

From (11), (12), (13), and (14), we obtain the analog for (8):

$$M_2 = q + 2q^2 + 7q^3 - 28q^4 + 19q^5 \quad (15)$$

and hence, as for (10):

$$P_{2x} = (1 - M_2^z)^x. \quad (16)$$

4. Individualistic Model: Mixing

We now examine the probability of at least partial mixing of colonies, i.e., of some workers at least of one colony being compatible with some or all

workers of a neighbor. This situation includes complete fusion as well as cases where fusion is not possible due to the hostility of some workers in each colony.

We first obtain similar quantities to those derived previously:

$$H_{3os} = 1 - (1 - q)^3 \quad (17)$$

$$H_{3od} = 1 - (1 - 2q)^3 \quad (18)$$

$$H_{3es} = 1 - (1 - 2q)^3 \quad (19)$$

$$H_{3ed} = 1 - (1 - 3q)^3. \quad (20)$$

From (17), (18), (19), and (20), we obtain the analog of (8) and (15):

$$M_3 = q(9 - 36q + 75q^2 - 78q^3 + 31q^4) \quad (21)$$

and hence, as for (10) and (16):

$$P_{3x} = (1 - M_3)^x. \quad (22)$$

Discussion

1. Gestalt or Individualistic Model as a General Explanation

Which of our two models would be the better general explanation for innate colony-odor differences in social hymenoptera? We favor the Gestalt model as fitting this niche best. Firstly, we note that, in many species, mixing the workers of different nests alters the behavior of nestmates on the return of the removed workers to their parent colonies, clearly indicating an altered colony odor. Such an alteration is characteristic of the Gestalt, and not the Individualistic, schema. Secondly, many species have multi-queened colonies or multiple insemination, or both; such a colony makeup would very often lead to severe internecine strife under the Individualistic model, but not under the Gestalt model, unless inbreeding or other factors maintained very high levels of relationship.

It is possible that the Individualistic model is applicable to some species, and that it represents an earlier stage in the evolution of the recognition system. Haskins and Haskins (1950) found that worker ants of the key primitive genus *Myrmecia* attack conspecific aliens, added to the colony as pupae, when they eclose. These aliens would thus have a distinctive odor not subject to merging with the general odor of the colony. Such an interpretation must be regarded with caution, however, because it is now known (Imai et al., 1977) that *Myrmecia* species are often clusters of siblings, so that the alien workers involved may *not* have been 'conspecific.' Even so, of course, the experiment demonstrates a greater degree of pheromonal integrity than present in many other ants.

We also note a final, perhaps less compelling, reason for preferring the Gestalt model: the Individualistic model can only operate under a male-haploid or sex-linked genetic system. Colony odor is well known among termites: unless any genetic components are determined only by sex-linked loci, the Individualistic model cannot apply to them for the same reason that it cannot apply to multiply inseminated ant species: internecine strife would destroy many colonies.

Table 1. Number of alleles per locus required to achieve distinctness of a colony from all of one, five, or ten neighbors with a probability of 0.95. See text for further discussion of the models and assumptions involved

Number of loci	Number of alleles								
	Gestalt, no fusion			Individualistic					
				No fusion			No amicability		
1	4,	6,	8	23,	101,	198	176,	877,	1754
2	2,	3,	3	7,	12,	17	37,	85,	122
3	2,	3,	3	5,	7,	8	21,	38,	49
4	2,	2,	2	4,	5,	6	15,	24,	30
5	2,	2,	2	3,	4,	5	12,	19,	22
6	2,	2,	2	3,	4,	4	11,	15,	18
7	2,	2,	2	3,	4,	4	9,	13,	15
8	2,	2,	2	3,	3,	4	9,	12,	13
9	2,	2,	2	3,	3,	3	8,	11,	12
10	2,	2,	2	2,	3,	3	7,	10,	11
11	2,	2,	2	2,	3,	3	7,	9,	10
12	2,	2,	2	2,	3,	3	7,	9,	10
13	2,	2,	2	2,	3,	3	6,	8,	9
14	2,	2,	2	2,	2,	3	6,	8,	9
15	2,	2,	2	2,	2,	3	6,	8,	8

2. How Many Loci Are Involved?

Could innate colony odor be due to a single pheromone, or is it most likely that a number of pheromones, produced by different loci, is involved? We approach this question, posed by Barrows et al. (1975), who favored a multiplicity of pheromones, by asking how many alleles per locus would be required to give a level of colony distinctness compatible with observations. We chose a 95% probability of distinctness of a colony from all its neighbors as being a reasonable level, and used (10), (16), and (22) to compute Table 1.

From Table 1, we conclude that a battery of closely related molecules whose variation is controlled at one locus, and which function as pheromones, could indeed explain innate colony odor under the Gestalt model, in that only six alleles are required when a colony has, on average, five neighbors. A single-locus situation seems much less likely for the Individualistic case: not only is a greater number of alleles required to prevent complete fusion, but the number required to prevent amicability of occasional foraging workers from different nests is much greater.

Even if it is possible for a single pheromone to function to maintain colony distinctness, it may well be that, in fact, a number do so. Pheromones with other functions may, through allelic diversity, also function to give colonies distinctive odor. The number of loci involved in colony-odor production can be gauged through inbreeding. Thus, in species whose colonies are founded by single, once-mated queens, the males and young queens comprise an F1 generation; sib mating these individuals gives rise to an F2. The probability

of fusion of colonies headed by F2 queens derived from the same F1 queen will be much higher than that for outbred colonies. Assuming that colonies have, on average, five neighbors, and that for 95% of the time they are incapable of fusion with all five, the pairwise tests of the colonies headed by sister F2 queens could enable distinction between one locus or many as the basis for innate colony odor. Thus, under our assumptions for the Gestalt model, the overall probability of compatibility for z loci is:

$$P_{cg} = ((1 + 3q)/4)^z. \quad (23)$$

If, for example, one locus is involved, then from Table 1, six alleles are required for a 95% distinction probability of colonies from all neighbors in the original population, and the probability of compatibility between two colonies headed by sister F2 queens is, from Eq. (23), 0.38. However, if ten loci are involved, with two alleles at each, then the probability of compatibility drops to 0.01. For the Individualistic model, the analogous equation to (23) is;

$$P_{ci} = ((1 + 2q(2 - q))/3)^z \quad (24)$$

in which case, for one locus with 12 alleles, the probability of fusion is 0.44, whereas with ten loci, with three alleles each, this probability is 0.03.

Final Remarks

For the purpose of fixing ideas, we treated allelic diversity at the colony-odor loci as involving the production of different molecules within the colony. However, as Barrows et al. (1975) suggest, different odorant concentrations might be involved. Such a model would not differ in principle from the one we have presented, although there would be differences in detail in the subsequent analysis. An additional possibility is that a queen-produced pheromone may also contribute to colony odor. The queen contribution may be the most important in young colonies, but be swamped by that of the workers in full-sized colonies.

Inasmuch as colony odor enables individuality of the colonial 'superorganism,' colony-odor loci invite comparison with vertebrate histocompatibility antigen loci, which have been suggested as having arisen from selection for individual distinction mechanisms (Burnet, 1973). Burnet (1973) suggests that histocompatibility antigen loci arose through selection preventing intraspecific parasitism, and the vulnerability of social insect colonies to robbing by others of the same species, as in honeybees, raises an obvious parallel. Naturally, there are significant differences between the two situations, as well as the similarities indicated, with these differences arising from the fact that vertebrate individuals are clonally derived, whereas social insect colonies are not.

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