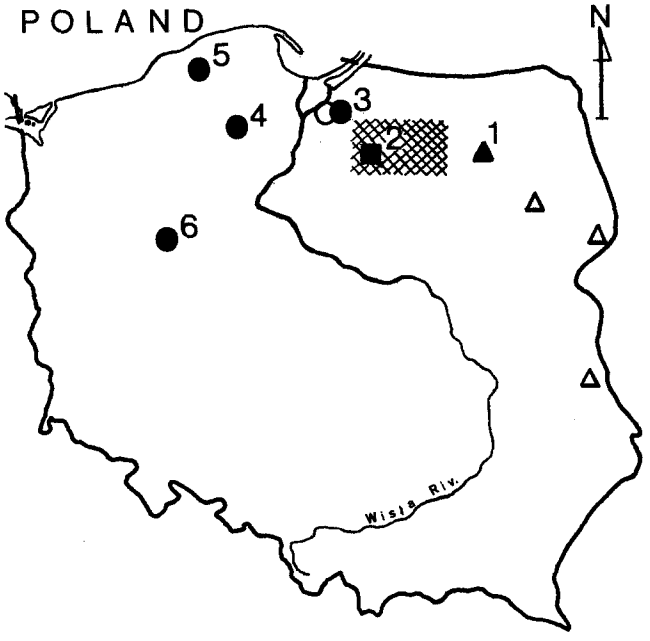

Figure 6. Distribution of karyotypic races of *S. araneus* in Poland. Triangles: eastern races, circles: western race, square: probably a hybrid race, shading: region of contact between the eastern and western races. Open symbols: data from earlier papers[12,13], and closed symbols: present data. Numbers of localities as in table 1.

fore the emergence of the 'Łęgucki Młyn' race can be explained as follows: The first step was the reciprocal translocation of arms in metacentrics *hi* and *ko* from the western race, which resulted in the arms combinations *hk* and *io*. Then the second step would have involved the appearance of the arms combinations *gr* and *mn* as in the 'Popielno' race, as the result of the contact with eastern races.

The foregoing justifies the statement that in northern Poland the contact zone between the eastern and western karyotypic races of *S. araneus* occurs in the region defined by coordinates E 19°30–E 21°30 (fig. 6). Further studies will improve the knowledge of more precise boundaries of this contact zone crossing through Poland. More data gathered by the author, not yet published, suggest that the greater part of Poland is inhabited by shrews of the western race, and only the eastern and north-eastern fringes are occupied by shrews of the eastern races.

1 I wish to express my gratitude to my wife, A. M. Wójcik, M. Sc., for her help in catching the animals and making chromosome preparations. I must also offer my thanks to Dr J. Zima for giving me access to his as yet unpublished data, and to Prof. Z. Pucek and Dr S. Fedyk for reading the first draft of this paper.
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The cloning of more highly productive fungal strains: a factor in the speciation of fungus-growing ants[1]

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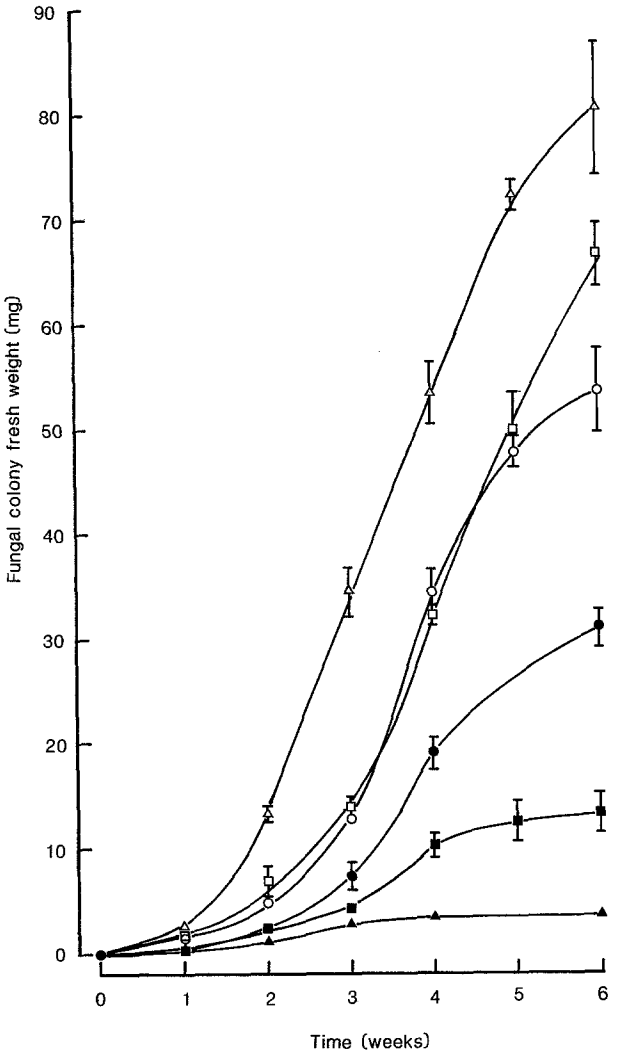
Summary. Paired-culture tests and productivity estimates were made on isolates of fungal symbionts from four species of attine ants. No interactions between different isolates in paired-cultures were observed. Significant differences in productivity were recorded between all isolates. A strong correlation was found between fungal productivity and mature colony worker population of the respective symbiotic ant species.
Key words. Ants; Attini; fungi; symbiosis; cloning; productivity; populations; speciation.

The New World myrmicine tribe Attini contains some 190 species in 11 genera which cultivate fungi as a source of larval food. The relationship is one of obligate symbiosis both for ants and fungus. Several features, including mature ant colony size and the material used as substrate for fungus cultivation, suggest an evolutionary trend. Whereas primitive genera with small colonies use insect frass and dead vegetable material, advanced types such as *Acromyrmex* and *Atta*, cut fresh plant material and their colonies may attain vast size[2] and pest status. New attine colonies are established by claustral foundation. Prior to the nuptial flight each gyne secures a sample of mycelium in the infrabuccal pocket[3] and later, as a foundress queen, uses it to establish a new culture. From this, future generations of gynes will in turn derive their own inocula. The fungus cultivated by attines is characterised by the production of small globular bodies known as gongylidia which constitute the principal supply of larval food[4]. Chemical analysis has shown the mycelium to be a nutritious food, rich in amino acids and carbohydrates[5].

The identity of the fungus cultivated by the higher attines remains obscure. It competes poorly with other fungi, and, on the removal of the ants, their cultures are rapidly overrun. Competition from fungal contaminants is eliminated by the secretion of β-hydroxydecanoic acid (myrmicacin) which has been shown to suppress spore germination[6]. Records of taxonomically essential sporophores derived from mycelia associated with advanced attines are few, and their true origins in doubt due to the high probability of contamination and the failure of authors to propagate gongylidia-bearing mycelia from the spores. Most recent authors have chosen to use the name *Attamyces bromatificus* Kreisel. This is based on descriptions of the symbiont of the Cuban *Atta insularis* which in the absence of diagnostic sporophore characters was placed in the Mycelia Sterilia[7]. We have recently[8] examined mycelia from 12 attine species and subspecies

in 5 genera all of which were found to have dolipore septation thus establishing their Basidiomycete identity. The absence of any sexual or sporulating stages and the ants' vegetative propagation of their gongylidia-producing mycelia suggests that these fungi are closely related. The readiness of some attine species to accept each other's cultures has also been taken as evidence of fungal relatedness[4]. In laboratory PDA cultures, optimum growth of fungal symbionts from all the attine species considered here was obtained under closely similar conditions of temperature and pH[8].

Materials and methods. Isolates of attine fungi were obtained from the following sources; *Trachymyrmex urichi* Forel, *Acromyrmex octospinosus* Reich and *Atta cephalotes* L. from Trinidad; *A. sexdens sexdens* L.[1] from Guyana; *A. sexdens rubropilosa* Forel[1] from both Brazil and Paraguay. All isolates were cultured on PDA plates buffered at pH 5.0 and incubated at 25 °C in darkness[8]. A series of culture plates were inoculated with pairs of different isolates representing all 15 possible combinations, each combination being replicated three times. These were incubated until the mycelia of both inocula had grown together and then inspected for signs of interaction. A further set of 24 replicate plate cultures were established from each of the six isolates alone and incubated for periods of up to six weeks. Total fungal fresh weight, and gongylidia biomass were determined[8] for four plates from each isolate at weekly intervals.



Total production (± SE) by PDA cultures of fungal isolates from different attines during a period of six weeks. (▲) *T. urichi*, (■) *Ac. octospinosus*, (●) *A. cephalotes*, (○) *A. sexdens sexdens*, (□) Brazilian *A. sexdens rubropilosa*, (△) Paraguayan *A. sexdens rubropilosa*.

Table 1. Mean estimated gongylidia production (mg) after six weeks of incubation by PDA cultures of fungal isolates from attine ant colonies

Ant species	Source	Mean gongylidia biomass ± SE
Trachymyrmex urichi	Trinidad	1.72 ± 0.16
Acromyrmex octospinosus	Trinidad	6.49 ± 0.13
Atta cephalotes	Trinidad	13.10 ± 0.24
Atta sexdens sexdens	Guyana	33.49 ± 0.14
Atta sexdens rubropilosa	Brazil	41.47 ± 0.51
Atta sexdens rubropilosa	Paraguay	50.12 ± 0.40

Results. In all replicates of the 15 paired tests, mycelia of both isolates interdigitated freely without any signs of interaction. Neither clamp connections, indicating different mating strains, nor inhibition zones were observed. The increases in fungal fresh weight shown by the separate cultures (fig.) indicate clear differences in productivity between all six isolates by the sixth week of incubation. Total fungal production was reflected in the 'crop' yield of gongylidia (table 1). Comparison of gongylidia production after six weeks incubation with estimates of mature colony worker populations for the respective ant species (table 2) reveal a strong correlation, $r = 0.99$ ($p < < 0.001$) described by the linear regression; population = $-225874+70157 \times$ gongylidia biomass.

Discussion. Existing evidence suggests that the higher attines cultivate the same species of basidiomycete fungus. Although tests for fusion between different mycelia of any of the six isolates used in this study were not performed, the absence of obvious interactions in paired cultures supports this suggestion. Not only is the production of sporophores by these fungi doubtful but the secretion of myrmicacin by the ants militates against the involvement of spores in propagation. In the light of this evidence we would go further and propose that not only do different species of attine ants cultivate the same species of fungus but that through their vegetative method of propagation these cultures constitute fungal clones which may be shared by more than one ant species and therefore of great antiquity.

Despite the evidence for close similarity between fungal isolates from different attine species our results reveal significant differences in their productivity. Of particular interest is the correlation between fungal productivity and the potential maximum colony population of the ant species concerned. Thus *Atta cephalotes*, whose colonies reach worker populations of 0.7×10^6, cultivates a strain of fungus whose productivity is 7.62 times that of *Trachymyrmex urichi*, a species with colonies of up to about 800 workers. *Acromyrmex octospinosus*, with colonies of up to 14×10^3 workers, cultivates a fungus with intermediate gongylidia productivity, 3.77 times that of *T. urichi*. Isolates from *Atta sexdens rubropilosa*, whose colonies are estimated to attain worker populations of 3×10^6 cultivate the most productive strain we tested, isolates of Paraguayan origin yielding a mean of 50.12 ± 0.4 mg of gongylidia in 6 weeks, almost 30 times the yield from *T. urichi* fungus.

Table 2. Estimated sizes of mature attine colonies

Ant species	Maximum worker population	Source
Trachymyrmex urichi	763 ± 76	Weber (1972)[4]
Acromyrmex octospinosus	14,278	Lewis (1975)[9]
Atta cephalotes	0.7×10^6	based on Weber (1972)[4]*
Atta sexdens sexdens	2.2×10^6	Weber (1966)[3]
Atta sexdens rubropilosa	3.0×10^6	Dias[1]

* Derived by applying Weber's direct count of 8762 workers in a representative fungus garden of this species to our own observation of a mature field colony containing 80 full sized fungus gardens.

The gongylidia constitute the larval diet and so the rate at which they are produced might be expected to have significant importance to the establishment success of foundress queens, growth of worker populations and the production of sexuals. Larger colony worker population would allow an increased foraging range and thus facilitate a qualitative and quantitative expansion of the trophophoric field. Thus the acquisition of a more productive food crop would confer a selective advantage in permitting the exploitation of richer and more abundant substrate resources and would therefore constitute an important factor in attine niche separation. In the absence of sporulation, the only source of more productive fungal strains would be through somatic mutation. We suggest that ancestral selection of high yielding somatic mutants from their cloned fungal symbionts was a major factor in the speciation of the higher attines allowing niche separation through selection for increased queen fecundity and colony population.

In support of this hypothesis is our observation of a declining colony of *T. urichi* whose fortunes were dramatically reversed after providing it with fungal material taken from *Atta cephalotes*. Within a period of 12 months not only did the worker population rise above that of two other colonies of the same species but large numbers of sexuals were produced. Subsequent plate culture of isolates verified that it had indeed adopted the more productive fungal strain.

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Acyl fluorides as reactive mimics of aldehyde pheromones: hyperactivation and aphrodisiac in *Heliothis virescens*[1]

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Summary. Substitution of fluorine for the aldehydic hydrogen provides behaviorally active, chemically reactive pheromone mimics. In male moths of the tobacco budworm *Heliothis virescens*, Z9–14:Acf and Z11–16:Acf cause hyperactivity and irreversible extension of the genitalia in over 80% of treated moths. In addition, a combination of the two components leads to 10–50% of the pairs involving one treated partner becoming locked in copula.
Key words. Acid fluoride; aldehyde; mating disruption; pheromone analogs; *Heliothis;* Lepidoptera; fluorination.

The use of pheromones and pheromone mimics for the suppression of insect populations by mating disruption is becoming practical and environmentally acceptable[2–4]. Substantial quantities of pheromones are required for this management strategy, since saturation of the atmosphere over large areas (> 3 ha) for several days and nights must be achieved to effectively reduce mating. An alternative approach, where insect pheromone receptors would be stoichiometrically and irreversibly modified, would in principle require much less chemical and shorter exposure times. We now report the synthesis of two acyl fluorides which mimic the structures of the two major aldehyde components of the pheromones of *Heliothis* species. With *H. virescens*, these acyl fluorides elicit aberrant sexually-oriented behavioral responses in male moths which suggest the involvement of a novel form of sensory disruption based on receptor modification.

The tobacco budworm, *Heliothis virescens*, uses a 15:1 blend of (Z)-11-hexadecanal (Z11–16:Ald) and (Z)-9-tetradecenal (Z9–14:Ald) as the behaviorally important components[5] of a blend of seven aldehydes identified from female glands[6,7]. The importance of the minor aldehyde and alcohol components in eliciting complete mate-finding and mating behavior by *H. virescens* males has been demonstrated in recent wind-tunnel experiments[8]. Formates[9] and diolefins[10] have been employed as stable aldehyde analogs for mating disruption of *H. virescens* and *H. zea*, and give significant reductions in field populations of these important pests. We envisioned the use of (Z)-11-hexadecenoyl fluoride (Z11–16:Acf) and (Z)-9-tetradecenoyl fluoride (Z9–14:Acf) as reactive analogs of the *H. virescens* pheromone aldehydes. Precedent for this analogy is found in the use of retinoyl fluoride as a mimic of retinal to give specific inactivation of bovine opsin[11]. The inactivation is due to the irreversible formation of a covalent amide adduct with the primary amino group of opsin, instead of the normal reversible Schiff base formation which occurs to give rhodopsin. We postulated that aldehydes might interact with primary amino groups of a putative receptor protein in a 'fast-and-loose' Schiff base formation[12]. Protein conformational changes could then mediate the transduction of the olfactory stimulus into a nerve impulse[13] and resulting behaviors. With this simple model, an acyl fluoride

Number of *H. virescens* moth pairs locked in copula (L) or dead (D) after 5 h exposure to 2 mg of acyl fluoride(s) on a paper wick. Seven moth pairs were evaluated in each of four possible crossed mating combinations (T = treated, N = not treated). Mating success as total spermatophores from 7 ♀ is also shown

		T♂ × T♀	T♂ × N♀	N♂ × T♀	N♂ × N♀
Z9-14:Acf	Day 1	1 L	0 L	0 L	0 L
	Day 4	3♂, 1♀ D	1♂, 1♀ D	1♂, 1♀ D	0 L, 0 D
	Total spermatophores	15	17	17	15
Z11-16:Acf	Day 1	0 L	0 L	0 L	0 L
	Day 4	1 L, 0 D	1 L, 0 D	2 L, 0 D	0 L, 0 D
	Total spermatophores	18	13	17	15
Z9-14:Acf	Day 1	3 L	4 L	1 L	0 L
	Day 4	1♀, 3♂	1♀, 2♂ D	2♀, 1♂ D	0 L, 0 D
+ Z11-16:Acf (1:1)	Total spermatophores	9	13	7	14