Social cancer and the biology of the clonal ant *Pristomyrmex punctatus* (Hymenoptera: Formicidae)

Kazuki TSUJI & Shigeto DOBATA

Abstract

We review some aspects of the biology of the ant *Pristomyrmex punctatus*, in which the winged queen caste is absent and wingless females reproduce by thelytokous parthenogenesis. The majority of females have two ovarioles, whereas up to 50% of colonies contain large-bodied females which have four ovarioles. We call the former workers and the latter ergatoid queens. Males are rare. Some ergatoid queens have a spermatheca, but no inseminated individual has been found so far. Castes are morphologically defined, and workers engage in asexual reproduction in all colonies regardless of the presence of ergatoid queens. In colonies containing only workers, reproductive division of labor is regulated by age-polyethism: All young workers reproduce and fulfill inside-nest roles, and old workers become sterile and fulfill outside-nest roles. Colonies are founded by fission or budding, and consequently neighboring colonies are often related. Nevertheless, populations are multi-colonial, with strong hostility among neighboring colonies. A genetic analysis revealed that colonies often have multiple genotypes (parthenogenetic lineages), and suggested that the majority of those lineages can produce both workers and ergatoid queens. However, a lineage in a population in central Japan produces only ergatoid queens. We define these queens as cheaters, as they fulfill no other task than oviposition and therefore depend on the work force of other non-cheater lineages. Ergatoid queens in cheater lineages have three distinct ocelli, but those in non-cheater lineages usually have zero to two. As cheaters are likely to be horizontally transmitted, we draw an analogy to transmissible cancers. The coexistence of cheaters and non-cheaters is discussed in the frameworks of multilevel selection in the short term, and local extinction-immigration in the long term. However, many things remain to be studied, such as the developmental origin of the ergatoid queens, the frequency of sexual reproduction, and how colony identity is maintained.

Key words: Clonal ant, cheating, social cancer, genetic caste determination, parthenogenesis, ergatoid queen, colony discrimination, review.

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Introduction

In queenless ant species, the role of morphologically distinguishable queens as the primary reproductive females is taken by mated workers called gamergates. Gamergates in some members of the Amblyoponinae, Ponerinae, and Ectatomminae are functional queens (MONNIN & PEETERS 2008), and colonies show reproductive division of labor between gamergates and sterile workers. In contrast, although winged queens are also absent in the myrmicine ant *Pristomyrmex punctatus* (previously called *P. pungens*) and in the cerapachyine ant *Cerapachys biroi*, the reproductive division of labor is unrelated to mating (TSUJI 1988a, RAVARY & JAISSON 2004). Instead, many wingless females reproduce totally asexually by thelytokous parthenogenesis (ITOW & al. 1984, TSUJI & YAMAUCHI 1995). These social systems differ from those recently found in two, also obligatory parthenogenetic, myrmicine ants, *Mycocepurus smithii* and *Pyramica membranifera*, in which winged queens produce both sterile workers and new queens completely parthenogenetically (HIMLER & al. 2009, RABELING

& al. 2009, ITO & al. 2010). In this paper, we review some aspects of the biology of *Pristomyrmex punctatus* to clarify its peculiarity among "thelytokous ants". We also discuss that *P. punctatus* is a good system to study evolutionary dynamics of cooperation and cheating.

Life cycle and division of labor

The majority of colonies in *Pristomyrmex punctatus* consist of only workers that are morphologically defined (TSUJI 1988a, 1995), although some colonies have ergatoid queens whose definition is described later in detail. First, we describe the biology of those colonies containing only workers. In ants and social bees, division of labor among workers is mostly age dependent, in that young workers fulfill inside-nest roles such as nursing, while old workers perform outside-nest tasks such as foraging (the temporal castes or age-polyethism; OSTER & WILSON 1978). In *P. punctatus*, reproductive division of labor is regulated by this age-polyethism (TSUJI 1990a): Young workers reproduce and

simultaneously perform other inside-nest tasks, while old workers cease reproduction and perform outside-nest tasks. The distribution of the number of mature oocytes that nest-workers have fits a Poisson distribution (TSUJI 1988a). This means that all young workers have a similar probability of egg-laying and the variation of short-term fecundity may occur just by chance. All workers follow this same behavioral development, and therefore there are neither permanently reproductive nor permanently sterile workers. Furthermore, neither dominance behavior nor policing has been observed among workers (TSUJI 1988a, 1990a). This situation has raised semantic arguments of whether this ant should be regarded as eusocial or not (TSUJI 1990a, 1992, FUREY 1992). Reproductive division of labor in *Cerapachys biroi* is likewise regulated by age-polyethism. Although *C. biroi* colonies show cyclic phases as seen in some *Eciton* army ants (RAVARY & JAISSON 2002, RAVARY & al. 2006), old workers do not reproduce even in the stationary (non-migratory) phase, in which young workers lay eggs (RAVARY & JAISSON 2004).

The reproductive biology of *Pristomyrmex punctatus* and *Cerapachys biroi* markedly differ from any other example of ants that are known to be able to reproduce by thelytokous parthenogenesis in that the majority (possibly all) of females participate in reproduction at least once in their life. In these other ants, the majority of females are sterile helpers, with reproductive division of labor regulated either by the physical caste system (such as in *Anoplolepis gracilipes*, see DRESCHER & al. 2007, *Cataglyphis* spp., see PEARCY & al. 2004, TIMMERMANS & al. 2008, *Pyramica membranifera*, see ITO & al. 2010, *Mycocepurus smithii*, see HIMLER & al. 2009, RABELING & al. 2009, *Messor capitatus*, see GRASSO & al. 2000, *Vollenhovia emeryi*, see OHKAWARA & al. 2006, KOBAYASHI & al. 2008, and *Wasmannia auropunctata*, see FOURNIER & al. 2005) or by behavioral dominance and / or policing (in *Platythyrea punctata*, see HARTMANN & al. 2003). Furthermore, *Pristomyrmex punctatus* and *Cerapachys biroi* contrast with *Platythyrea punctata* in which either physical caste system or behavioral interference regulates reproductive division of labor depending on colonies and populations (HEINZE & HÖLLDOBLER 1995, SCHILDER & al. 1999, HARTMANN & al. 2003).

Although age-polyethism is the general pattern that in turn regulates the reproductive division of labor in *Pristomyrmex punctatus*, individuals show large variation in behavioral development: The timing of the switch from inside-nest tasks to outside-nest tasks occurs two to three weeks before the worker's death (in lab-reared colonies), yet individual life span is highly variable for unknown reasons (TSUJI 1990a). Consequently, a long-lived worker tends to have a longer inside-nest phase and therefore more opportunity for reproduction than a short-lived one. At least two interpretations of the adaptive significance of this phenomenon are possible: either that the long lifespan in some workers is a manifestation of the individuals' self-interest (see our later discussion on cheaters), or that this is a means to maintain the colony's efficient task allocation. The annual reproductive cycle of colonies we studied in central Honshu, the main island of Japan, shows why such flexibility would be needed: New adults emerge from June to September (peaking in early August). They withhold reproducing until after overwintering. Overwintered adults begin oviposition in April; they lay eggs and raise brood until the end of fall, when they die. The adult emergence period (June – September) is shorter than the breeding period (April – September). Therefore, if all individuals followed the same schedule of age-polyethism, inefficient task allocation would happen at some time of the year; for example, a shortage of foragers or of egg-layers. Such unbalanced task allocation lowers colony efficiency under experimental conditions (TSUJI 1994) and probably also in the field (TSUJI 1995).

Individual variation might enable a colony to evade such a problem. TSUJI (1994) found that laboratory colonies of which all foragers were removed at the outset of the experiment later produced some brood. Indeed, during the experimental period for 20 days, some young workers which had never previously foraged started to forage and obtained food. Although this behavior might have been an artifact caused by the small size of the lab cage, or more simply aging (some might have become foragers) could account for it, it is also possible that some nest-workers could be flexibly recruited to foraging when a large work force is needed. So far, division of labor of this species was observed mostly in laboratories with constant conditions. Behavior, in particular the flexibility of division of labor, should be studied also under heterogeneous environments like in the field.

Migration, colony founding, and nestmate discrimination

Despite its unusual biology, *Pristomyrmex punctatus* forms a typical multi-colonial population (TSUJI & ITÔ 1986). The colony size ranges from 4,000 to 320,000 adults (TSUJI 1988a, 1995). Neighboring colonies are strongly hostile toward each other, and lethal fights sometimes occur in the field (TSUJI 1988b). The absence of alate queens implies that new colonies must be founded by fission or budding. However, so far no one has directly observed a colony being founded in the field. In laboratories, small colonies, with less than 100 individuals, always show very low performance and often fail to produce any adult offspring (K. Tsuji & S. Dobata, unpubl.). This suggests that colony founding should occur with a large group of individuals, i.e., possibly at least hundreds. Colonies do not construct an elaborate underground nest, but instead use naturally pre-existing cavities, such as the space under a stone. They frequently move nest sites, and the average residence time is around two to three weeks (TSUJI 1988c). Colonies are usually monodomous, but become polydomous under some conditions (TSUJI 1988c). Polydomous colonies are more frequently found in summer, when they actively forage, than in spring and fall, when foraging is less active (TSUJI 1988c). Colony fission is inferred to occur by chance as a consequence of the frequent migrations, which can also occasionally give rise to polydomy (TSUJI 1988c).

Neighboring colonies of fission-founding ants are likely to be related. An isolation-by-distance pattern revealed by genetic markers empirically supported this prediction in *Pristomyrmex punctatus* (see DOBATA & al. 2011). This situation raises the question of nestmate discrimination: How can these ants discriminate colony membership so strictly (TSUJI & ITÔ 1986, TSUJI 1988b) despite the genetic similarity? They may use environmentally derived chemicals as colony-specific labels (TSUJI 1990b). Indeed, artificial mixing of different colonies and thus of the putative

Tab. 1: A list of names previously used to describe phenotypic variants of *Pristomyrmex punctatus* females. * + is present, – is absent, ? means information insufficient or non-available (GOTOH & al. 2011). ** TERANISHI (1929) wrote that the ergatoid queens he defined can be also called pseudogynes. *** In non-cheater lineages, ocelli (if any) are always vestigial and can be observed only by using strong light that passes through the ant's head (DOBATA & al. 2011). Therefore, it is not surprising that many previous authors did not recognize them.

Phenotype			Description of its occurrence	References and used terminology			
Number of ovarioles	Number of ocelli	Sperma-theca*		This paper, DOBATA & al. (2011)	WANG (2003), ITOW & al. (1984), TERA-NISHI (1929)**	TSUJI (1988a, 1995), SASAKI & TSUJI (2003)	DOBATA & al. (2009)
2	0	–	More than 50% of colonies consist of only this female category.	worker	worker	small (or normal) worker	S-type
4	0	– (?)	Found in 5 - 50% of colonies in all populations. Its frequency varies over years. Its intracolonial proportion is 0 - 30%.	ergatoid queen	(no name is provided)	large worker	L-type
	1-2	?			(no name is provided) ***		
	3	+	Very rare. So far, collection is repeatable only in 2 populations. Its intracolonial proportion is 0 - 50%.		ergatoid queen		

labels indicated that ants can absorb labels from outside (TSUJI 1990b). Regardless of whether the colony label is genetic or environmental, one can infer that neighboring colonies tend to share more similar labels with each other than with distant colonies. Therefore, one could predict a positive correlation between geographical distance and intercolonial hostility, like the "dear enemy" phenomenon (FISHER 1954) that is known also in ants (e.g., HEINZE & al. 1996). However, SANADA-MORIMURA & al. (2003) observed the opposite in *P. punctatus*: Ants strongly attacked their neighbors but ignored ants from distant nests! The proximate behavioral explanation is that individual ants become more aggressive to a specific foreign colony because of frequent contact with members of that colony (SANADA-MORIMURA & al. 2003): Outside the nest, ants should more frequently meet neighbors than members of a distant nest under natural conditions. Such a flexible response can be adaptive, because neighbors should directly compete for resources such as food and nest sites, and thus pose a threat, but a worker from a distant nest is less likely to recruit colony members en masse and is therefore less of a threat. These phenomena might be more or less general in group-foraging ants (see also BROWN & GORDON 1997). These mechanisms assume that ants can discriminate nestmates from non-nestmates, but do not attack those from distant nests. However, colony-discrimination mechanisms in *P. punctatus* still largely remain to be studied.

NISHIDE & al. (2007) discussed the possible occurrence of colony fusion in a *Pristomyrmex punctatus* field population, because of the frequently observed intracolonial variation in microsatellite loci. However, we wonder about its generality for two reasons.

First, there is a frequent misunderstanding here: Asexual reproduction itself does not necessarily lead to a genetically homogenous colony or population unless experiencing a bottleneck in their specific lifecycle or in their entire population dynamics. High intracolonial genetic variation may simply reflect the long-lasting large effective population size (N_e) in each colony, because neutral theory predicts that a (nearly-)neutral mutant allele takes $4N_e$ generations to be fixed in a diploid population, and the mutation rates of microsatellite loci in *Pristomyrmex punctatus*

were estimated as ca. 10^{-5} per generation (DOBATA & al. 2011). In the colony's lifecycle of *P. punctatus*, there is seemingly no genetic bottleneck, such as the founding stage of a single queen, similar to the single zygotic stage of an embryo that grows into a multicellular organism. Therefore "somatic" mutations can build up within the colony. Note that this situation remarkably differs from that of another parthenogenetic species, *Platythyrea punctata*, in which behavioral interaction among workers in queenless colonies leads to monopolization of the colony's reproduction by a single parthenogenetic worker (SCHILDER & al. 1999, HARTMANN & al. 2003). Interestingly, for *Platythyrea punctata*, KELLNER & al. (2010) discussed that the loss of intracolonial genetic diversity is recovered by colony fusion.

Second, instead, high genetic differentiation among colonies within a local population is maintained in *Pristomyrmex punctatus*, contradicting the frequent occurrence of colony fusion. As measured by intracolonial relatedness (*r*), genetic differentiation among colonies was $r = 0.4325 \pm 0.0749$ (DOBATA & al. 2009). If colony fusions or intercolonial exchanges of individuals were frequent, such high genetic differentiation among colonies would not be expected. Alternatively, colony fusion could occur by chance when the colonies shared the same environmentally derived cues, but such a "hybrid" colony would soon be eliminated by colony-level selection. In fact, NISHIDE & al. (2007) experimentally revealed the low performance of genetically polymorphic colonies, shown as slower nest relocation and difficulty in aggregating, as is seen in chimeric slime molds (FOSTER & al. 2002), but this is unlike examples often discussed in other social insects in which high genetic diversity leads to high colony performance (e.g., MATTILA & SEELEY 2007, WIERNASZ & al. 2008). Many aspects of the maintenance of colony identity and the colony founding process remain to be studied.

Morphological castes and cheaters

In *Pristomyrmex punctatus*, the majority of adult females have two ovarioles (one in each ovary). In some colonies, females that have four ovarioles (two in each ovary) are also found. Different authors have used various terminologies for these morphological variants in *P. punctatus* (Tab. 1),
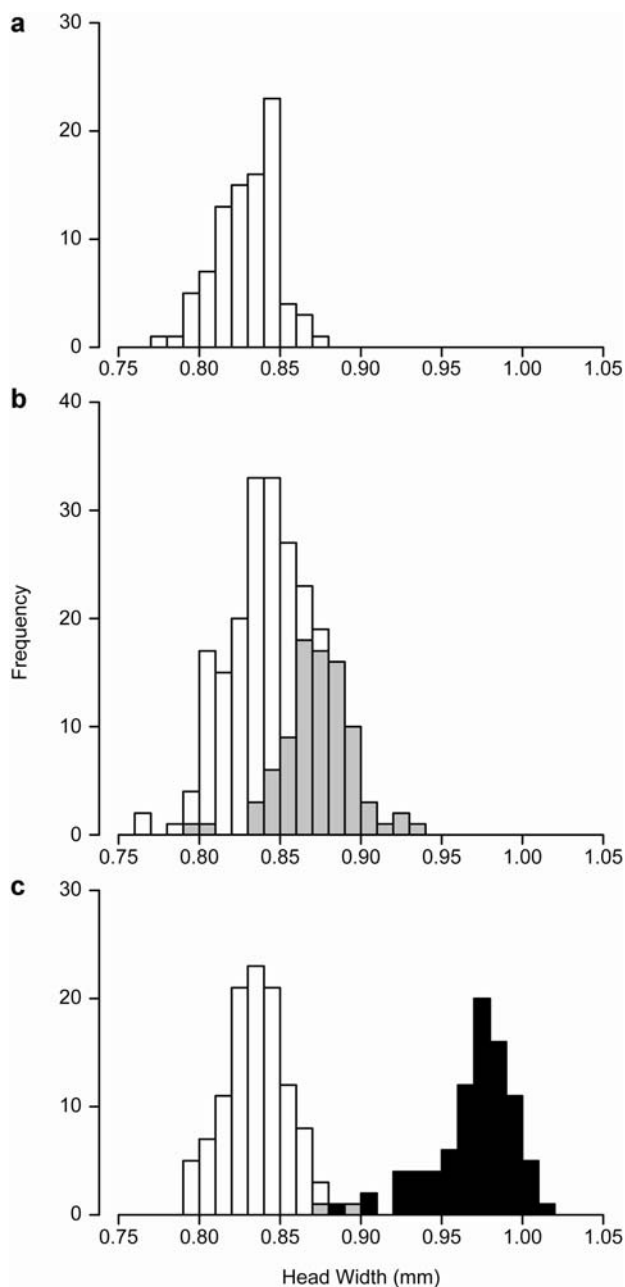
Fig. 1: The body-size distributions of workers and ergatoid queens in *Pristomyrmex punctatus* that were re-drawn using the data of DOBATA & al. (2009) studied in Kihoku population. (a) Colonies containing only workers (N[indv.] = 90; N[colony] = 9). (b) Colonies containing both workers and ergatoid queens with zero to two ocelli (N[indv.] = 228; N[colony] = 14). (c) Colonies containing cheaters (ergatoid queens with three distinct ocelli) (N[indv] = 198; N[colony] = 10). Workers, ergatoid queens with zero to two ocelli and cheaters are shown in white, grey and black bars, respectively.

causing confusion. Focusing on the discontinuity in the ovariole number and judging from morphology of workers and queens in related species, DOBATA & al. (2011) proposed calling the females with two ovarioles "workers" and those with four ovarioles "ergatoid queens" (permanently wingless queens as defined in MOLET & al. 2009). The use of the term "ergatoid queen" is justified also by the fact

that a portion of ergatoid queens, at least those with three ocelli (see later), have a spermatheca (ITOW & al. 1984, GOTOH & al. 2011). These morphological conditions in "ergatoid queens" of *P. punctatus* resemble ergatoid queens of other ants including some *Pristomyrmex* species (WANG 2003). However, we acknowledge that ergatoid queens in *P. punctatus* are exceptional, because they reproduce asexually (DOBATA & al. 2009, 2011), whereas most ergatoid queens known in other ants reproduce sexually (MOLET & al. 2009). Like in queenless colonies of other ants, *P. punctatus* workers keep reproducing asexually even in the presence of ergatoid queens (TSUJI 1988a, 1995). This is also an exceptional characteristic in *P. punctatus*.

The body size of ergatoid queens in *Pristomyrmex punctatus* is on average larger than that of workers, but with an overlapping distribution (Fig. 1b). Most ergatoid queens have zero to two ocelli, but some have three and are distinctly larger. Those with three ocelli are usually very rare, but are common in some populations. So far, the collection of ergatoid queens with three ocelli is repeatable only in two populations, i.e., Kihoku on Honshu island (DOBATA & al. 2009, 2011) and Takamatsu (Nomoto) on Shikoku island (HASEGAWA & al. 2011). Colonies containing many ergatoid queens with three ocelli have a bimodal distribution of body size (Fig. 1c; ITOW & al. 1984, SASAKI & TSUJI 2003, DOBATA & al. 2009), forming a striking contrast to the unimodal distribution of the majority of colonies (Fig. 1a, b). Ergatoid queens without ocelli can be found in most populations: depending on population and year, in up to 50% of colonies within a population, and up to 30% of individuals (TSUJI 1988a, 1995, DOBATA & al. 2011). The average proportion of ergatoid queens without ocelli in the entire population is usually 0.01 - 5% (TSUJI 1988a, 1995). However, a thorough dissection might detect ergatoid queens without ocelli at low proportions in most colonies. Colony size is not correlated with the proportion of ergatoid queens (TSUJI 1995). Much more rarely than those without ocelli, some ergatoid queens have one or two ocelli that seem more or less vestigial. The body-size distribution of these queens is in between those with three ocelli and those with none (DOBATA & al. 2009) (Fig. 2). We do not use the ocellus as the key character discriminating castes in *P. punctatus*, because we wonder about its function. In insects, ocelli function as light meters and are involved in stabilization reflexes during flight (KRAPP 2009); accordingly, they are often lost in non-flying insects such as workers of many ants. So ocelli are likely to be non-functional in ergatoid queens of *P. punctatus*.

Workers have no spermatheca, but ergatoid queens with three ocelli have a spermatheca with a seemingly normal accessory gland (GOTOH & al. 2011). Much less is known about the spermathecal condition in ergatoid queens with zero to two ocelli, though all ergatoid queens without ocelli examined so far (N > 200) apparently had no spermatheca (TSUJI 1988a, TSUJI 1995, SASAKI & TSUJI 2003, DOBATA & al. 2009).

Irrespective of the number of ocelli, ergatoid queens share the same behavioral characteristics: They lay more eggs than workers but rarely perform other tasks such as nursing (SASAKI & TSUJI 2003). Ergatoid queens were never observed to forage in the laboratory (SASAKI & TSUJI 2003), although they were occasionally collected on trails in the field (TSUJI 1988a). This suggests that ergatoid queens
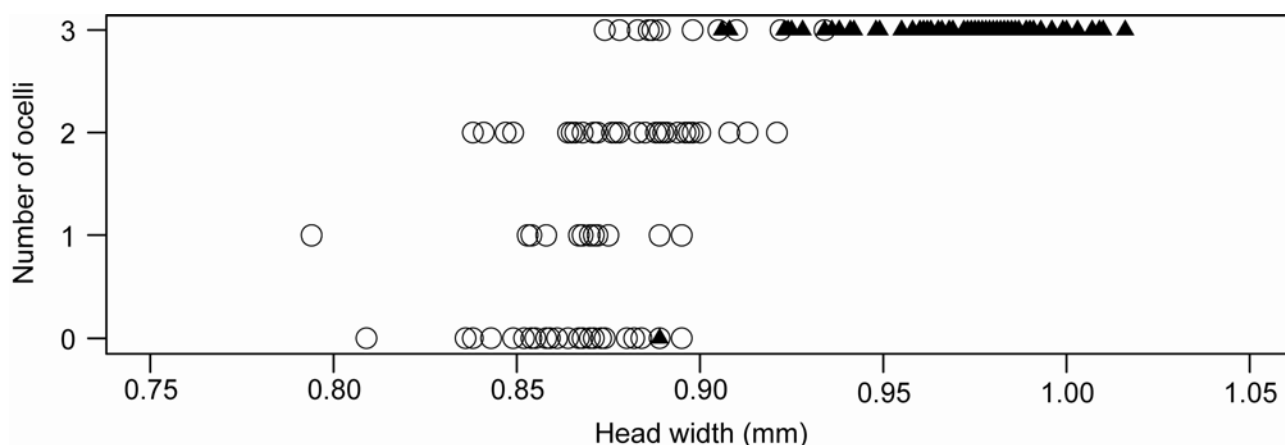
Fig. 2: The relationship between the body size (head width) of ergatoid queens and their number of ocelli. Data are from DOBATA & al. (2009) from the Kihoku population. Measures of cheaters (filled triangle) and non-cheaters (open circles) were combined. Correlation was positive and statistically significant (r = 0.760, p < 0.0001).

do not follow the age-polyethism and old individuals may continue to lay eggs. This behavioral propensity of ergatoid queens in *Pristomyrmex punctatus* is normal among queens of species in which the division of labor between reproductive queens and sterile workers enhances colony performance, but, as TSUJI (1995) discussed, ergatoid queens in *P. punctatus* could be harmful to the colony (see also SASAKI & TSUJI 2003) for the following reasons. Empirical evidence indicates that having only workers is enough for *P. punctatus* colonies to prosper (MIZUTANI 1980, TSUJI 1990a, 1994). More importantly, it is known that colonies with only workers are the most productive, and the average lifetime reproductive success of nestmates is estimated to decrease as the proportion of ergatoid queens increases in field colonies (TSUJI 1995). The contextual analysis applied to a field population detected no fitness peak (no stabilizing selection) at a small proportion of ergatoid queens (TSUJI 1995: tab. 4), suggesting that the idea that a low but non-zero ergatoid queen proportion in a colony enhances the colony's fitness through reproductive division of labor is not supported. Although we need more data across populations and years on the relationship between these individual and colony phenotypes and their reproductive outputs, their harmful effect to the colony is evident when ergatoid queens are genetically distinct cheaters as described below.

In Kihoku, central Japan, we found parthenogenetic lineages that give rise only to ergatoid queens (DOBATA & al. 2009). We defined these lineages as cheaters, because they can obtain a large reproductive output at the cost of fitness of other nestmate lineages that produce workers. Non-cheaters, or cooperators, are defined as lineages that can become workers. We use the plural term "lineages", because as mentioned previously, in *Pristomyrmex punctatus*, colonies are often genetically heterogeneous, particularly the colonies with cheaters always contain more than one genotype. This is owing to their social-parasitic characteristics, i.e., cheaters can exist only with workers that belong to a different lineage. In other words, cheaters depend on the work force of other lineages. Cheating in *P. punctatus* is therefore a genotypic concept, and is a specific case of genetic caste determination. A genetic analysis suggests that some non-cheater lineages produce only wor-

kers and other non-cheater lineages can produce both workers and ergatoid queens (DOBATA & al. 2011). The latter cases are most likely an account of phenotypic plasticity affected by larval nutrition as seen in the caste differentiation of most ants (DOBATA & al. 2011). Given the castes are mostly environmentally determined in those non-cheater lineages, we, however, do not rule out the possibility that some non-cheater lineages have a propensity to produce ergatoid queens. This is because such weak cheating is reported in some patrilines of a polyandrous *Acromyrmex* ant (HUGHES & BOOMSMA 2008), but is yet to be seen in *P. punctatus*. Interestingly, almost all adults of the cheater lineage (98.8%) examined so far have three ocelli. In contrast, ergatoid queens in non-cheater lineages have no or more or less vestigial ocelli that can be usually recognized only by using strong light that passes through the ant's head. Moreover, most of them have zero to two ocelli, and only 11.2% had three ocelli that are also more or less degenerated (DOBATA & al. 2011). Therefore, in practice, one can label ergatoid queens with three distinct ocelli as cheaters.

The monophyly of cheaters was suggested by analyses using microsatellite nuclear DNA and mitochondrial haplotypes in our study population in Kihoku (DOBATA & al. 2009, 2011). The existence of another cheater lineage of independent origin is inferred from mitochondrial DNA data in a population in Takamatsu, western Japan (HASEGAWA & al. 2011). However, as the rare occurrence of sexual reproduction in this species has been suggested (see later), further studies incorporating nuclear genomic data are needed. Cheaters of the identical multiloci genotype are found in many colonies. This occurrence, as also suggested by a more rigorous population genetic analysis (DOBATA & al. 2011), implies the horizontal transmission of cheaters. In other words, cheaters migrate to other intact colonies. Nothing is known about this process, however. Such horizontal transmission of cheaters despite the presence of strict nestmate discrimination is not surprising, because many social-parasitic species can evade the host's discrimination behavior. A parallel phenomenon is known in the Cape honey bee (*Apis mellifera capensis*), in which workers can reproduce by thelytokous parthenogenesis, as does *Pristomyrmex punctatus*. Some parthenogenetic worker lin-

eages of the Cape honey bee have become social-parasitic and migrated among captive colonies of the neighboring subspecies *A. m. scutellata*, causing mass extinction of the host colonies (NEUMANN & MORITZ 2002, DIETEMANN & al. 2007). One can draw an analogy also to the transmissible cancer cells that infect and kill the host, as found in some mammals (e.g., MURGIA & al. 2006, PEARSE & SWIFT 2006). For example, a facial cancer transmitted among Tasmanian devils (*Sarcophilus harrisii*) through biting now poses a serious threat to the persistence of the species. By this analogy, the parasitic Cape honey bee workers and the *P. punctatus* cheaters are called social cancers (OLDROYD 2002, DOBATA & al. 2009; but see KORB & HEINZE 2004 for the case of Cape honeybee).

A question arises about the cause of the difference in the long-term consequences between these two social cancers; one leads to mass extinction within a decade or so (the Cape honeybee) the other persists much longer (*Pristomyrmex punctatus*). DOBATA & al. (2011) discussed that difference in migration ability of social cancers may account for the differential consequences. In theory, it is well established that limited migration of parasites can contribute to the persistence of host-parasite systems by creating the local extinction-recolonization process. The parasitic Cape honey bee workers have wings and thus can migrate further than the cheaters in *P. punctatus* that have no wings, which might hinder the former to persist long. Future theoretical and empirical studies should test this idea.

Similar to *Pristomyrmex punctatus*, *Cerapachys biroi* also shows a variation in body size and ovariole number among wingless females (RAVARY & JAISSON 2002, 2004), therefore some of these can be called ergatoid queens. More recently, LECOUTEY & al. (2010) revealed that colony demography and nutrition affects the production of ergatoid queens in *C. biroi*. However, if cheaters also exist in *C. biroi* or not is yet to be studied.

Parthenogenesis and rare sexual reproduction

ITOW & al. (1984) studied chromosomes in the ovaries of pupae of *Pristomyrmex punctatus* and revealed that the first meiosis occurs normally in developing oocytes. Microsatellite marker loci are often heterozygous (DOBATA & al. 2009). These results suggest that parthenogenesis in *P. punctatus* is a central-fusion-type automixis.

Although reproduction of this species is considered totally asexual, males are occasionally found. Males are haploid ($n = 12$; females have $2n = 24$) and have seemingly normal sperm (ITOW & al. 1984). As already mentioned, workers have no spermatheca, but ergatoid queens with three ocelli have a seemingly functional spermatheca (GOTOH & al. 2011). Therefore, ergatoid queens might occasionally reproduce sexually, although we have found no inseminated individuals despite having dissected more than a thousand ergatoid queens with three ocelli (T. Sasaki & K. Tsuji, unpubl.). In the Kihoku population, there is some mismatching between the mitochondrial DNA-based phylogeny and the nuclear microsatellite-based phylogeny, suggesting sexual reproduction in the past leading to genetic introgression among lineages (DOBATA & al. 2011). However, we stress that the dominant mode of reproduction is thelytokous parthenogenesis, which is indicated by several population-genetic indices; for example, the observed redundancy of the same multilocus genotypes due to distinct

sexual reproductive events has at most a probability of 0.8%, which indicates that this redundancy is derived mainly from thelytokous parthenogenesis (DOBATA & al. 2011).

Multilevel selection for the short-term dynamics of cheater and non-cheater populations

Although we previously mentioned that the long-term coexistence of cheaters and non-cheaters in *Pristomyrmex punctatus* can be described as a local extinction and immigration dynamics, another approach might be possible to understand their short-term dynamics. Ross Crozier was the first to point out that multilevel or group selection can be a useful framework to understand the maintenance of cooperative societies in this ant (see pages 99 - 100 in Discussion by ITOW & al. 1984 that was written by RC). TSUJI (1995) also discussed how the coexistence of non-cheaters and cheaters can be explained by multilevel selection. Individual selection favors cheaters, whereas colony-level selection favors non-cheaters. A balance of these two forces operating in opposition may lead to coexistence at least in the short term. Note, however, that group selection and kin selection are not alternative mechanisms but rather different descriptive models for the same phenomenon of evolutionary dynamics (QUELLER 1992). In fact, multilevel selection in *P. punctatus* can be transformed to kin selection. Cheaters obtain a higher individual fitness than non-cheaters (minus c) but harm the fitness of nestmates (minus b). Therefore they can increase in the (meta-)population when relatedness is low: That is, a cheater is no more likely than by chance to interact with cheaters in the background metapopulation. In contrast, cheaters can decrease when relatedness is high: That is, a cheater is more likely than by chance to interact with cheaters in the metapopulation, leading to mutual exploitation. We are conducting long-term research to monitor the microevolutionary process of cheater-non-cheater dynamics in the field and to test both group selection and kin selection models (see TSUJI 1995 for the earliest study).

Intraspecific parasitism or parasitic species?

People often wonder whether cheaters and non-cheaters are different species, in which case prey-predator or host-parasite ecological dynamics could provide a more appropriate description of the phenomenon than group or kin selection. We consider this view to be wrong for three reasons: (1) The biological species concept is not applicable to parthenogenetic organisms, and thus we have to resort to other criteria such as phylogenetic distance. (2) Although cheaters and non-cheaters form separate clades in the genetic analysis of the Kihoku population (DOBATA & al. 2009, 2011), both are nested in a deeper clade of the morphological species *Pristomyrmex punctatus* among other populations of Japan. Thus, cheaters and non-cheaters in Kihoku are phylogenetically more closely related to each other than to *P. punctatus* found in Okinawa. If cheaters and non-cheaters were regarded as separate species, many cryptic species should be discernable within the morphological species currently called *P. punctatus*. This would not be a practical decision. (3) A model of the biological dynamics in *P. punctatus* that includes two parties – one parasitic, the other non-parasitic – might be called a metapopulation host-parasite ecological model if the two parties are regarded as distinct species. Or it might be called a multilevel selec-

tion or family-structured kin-selection model if they are regarded as the same species. However, mathematically, both models are in principle the same with regard to predicting the relative abundance of the two parties when one is exploiting the other. Based on these arguments, we conclude that the criticism claiming that the two parties might be different species is semantic. Regardless of the taxonomic status of cheaters and non-cheaters, we believe that this system provides an ideal opportunity to test models of ecological and evolutionary dynamics.

The origin of cheaters

The body-size difference between workers and ergatoid queens (with three ocelli) in *Pristomyrmex punctatus* corresponds almost exactly to that of congeneric species with alate queens (DOBATA & TSUJI 2009). Furthermore, rudimentary wings can be seen under the cuticle of the thorax of these ergatoid queens. This evidence suggests that ergatoid queens in *P. punctatus* are real queens with arrested development.

However, we have to stress that there is an exceptional characteristic of ergatoid queens in *Pristomyrmex punctatus* and in *Cerapachys biroi*, in addition to their asexuality. Ergatoid queens in these two species reproduce together with many workers, forming a striking contrast to other cases in which reproductive division of labor between reproductive ergatoid queens and sterile workers is conspicuous.

The other congeneric species are all known to have queen (either winged or ergatoid) and worker castes (WANG 2003). Furthermore, males are found in many of those species (WANG 2003), suggesting their sexual reproduction. Therefore, asexual reproduction by workers instead of a winged queen in *Pristomyrmex punctatus* is likely a derived condition. Interestingly, *P. rigidus*, which is presumably the species most closely related to *P. punctatus*, exhibits a conventional social system with winged queens (WANG 2003). From the above circumstantial evidence we set out the hypothesis that cheaters in *P. punctatus* arose from a mutation that reuses the hidden developmental trajectory to a winged queen. In other words, although the ancestor of *P. punctatus* suppressed the queen trajectory, the genes encoding the developmental pathway are retained, and therefore mutations in regulatory regions can restore the pathway (except for full development of wings). Among such putative mutations, the ones with strong effects gave rise to cheaters in the Kuhoku population and the ones with weak effects lead to lineages producing both workers and ergatoid queens. Alternatively, the following scenario is also possible. Wing morphology of ant queens is likely an evolutionary labile trait (HEINZE & TSUJI 1995, MOLET & al. 2009, PERFILIEVA 2010). Indeed, a few species of *Pristomyrmex*, such as *P. africanus* and *P. wheeleri*, have both winged queens and ergatoid queens (WANG 2003). These intraspecific variation indicates winglessness of queens was evolutionary attainable also in *Pristomyrmex*. The ability of thelytokous parthenogenesis might have evolved in an ancestral species, where ergatoid queens reproduced sexually as in some other species of *Pristomyrmex* (WANG 2003). Then, parthenogenetic reproduction by ergatoid queens together with young workers prevailed. Later, in some populations, parthenogenetic queens became selected for, whereas in other populations ergatoid queens were selected against.

A comparative study across populations and across related species, with detailed descriptions of morphology and division of labor together with phylogenetic data, is needed to test these hypotheses.

Concluding remarks

Although the above scenarios on the origin of the unusual reproductive biology in *Pristomyrmex punctatus* are largely hypothetical, the ideas provide an interesting model system for future studies in developmental biology and in evolutionary biology from genomics to ecology.

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