

## Sexual and asexual reproduction of queens in a myrmicine ant, *Vollenhovia emeryi* (Hymenoptera: Formicidae)

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### Abstract

Sexual reproduction is more common than asexual reproduction in eukaryotes. There are few species with obligate clonality, and most clonal organisms maintain sexual reproduction. Organisms tend to couple sexual reproduction with dispersal, or changes in the environment. These facultatively clonal species use both reproductive systems to reduce the costs of clonality, and to gain benefits of sexuality. In recent decades, clonal reproductive systems have been discovered in some eusocial insects. In the myrmicine ant *Vollenhovia emeryi* previous research has documented that new queens are produced clonally with complete, diploid, maternal genomes. Usually, new queens have short rudimentary wings and cannot fly, suggesting that dispersal of new colonies is limited. However, some new queens with functional long wings occur in natural colonies, in addition to short-winged queens. In this study, we analyzed the reproductive modes of long-winged queens and short-winged queens. In agreement with previous data, most short-winged queens were produced asexually. However, mature colonies and nutritionally rich colonies tend to produce long-winged queens sexually. Since long-winged queens may encounter different environments after dispersal, higher genetic diversity should be advantageous to increase adaptability. Thus, *V. emeryi* may have evolved a reproductive strategy to maximize advantages of both clonal and sexual reproduction, as in other facultatively clonal organisms.

**Key words:** *Vollenhovia emeryi*, clonal reproduction, wing morphology.

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### Introduction

The evolutionary significance of sexual and clonal reproduction has been of great interest to biologists. Clonal reproduction is advantageous in stable habitats because genomes well adapted to the environment are not disrupted by sexual admixture and recombination (FELSENSTEIN 1974, BARTON & CHARLESWORTH 1998, WEST & al. 1999). In addition, clonality facilitates the rapid establishment of a species because there is no requirement for mating with partners (MAYNARD-SMITH 1971). However, it is widely accepted that clonal reproduction is likely to produce lower genetic diversity, and adaptation to environmental disturbances will be decreased. In addition, clonality causes an accumulation of harmful mutations in the genomes of individuals (MULLER 1964, FELSENSTEIN 1974, NORMARK & al. 2003). Consequently, species with clonal reproduction tend to retain the capacity for sexual reproduction for dispersal so as to minimize the risk of extinction (SCHEMSKE & LANDE 1985). Organisms using parthenogenesis, such as many plants, aphids, *Daphnia*, and many other facultatively clonal organisms, seem to use two reproductive systems conditionally

for non-dispersing and dispersing offspring (or offspring that are exposed to environmental change) (GOUYON 1999, SIMON & al. 2002, GREEN & FIGUEROLA 2005). These organisms tend to couple dispersal with sexual reproduction because genetic variation and higher adaptability should be required for survival in novel habitats (VAN VALEN 1973, WILLIAMS 1975), suggesting that these species maximize advantages of both reproductive systems.

Recent studies have shown that reproduction strategies are diversified in eusocial Hymenoptera, especially in ants (ANDERSON & al. 2008). Usually, diploid females are produced by sexual production, and castes (queens or workers) are determined by environmental or hormonal factors, whereas haploid males inherit only half of the maternal genome (DE MENTEN & al. 2005, SCHWANDER & al. 2008, LIBBRECHT & al. 2013). However, new examples of asexual reproduction have been discovered in ants (FOURNIER & al. 2005, KOBAYASHI & al. 2008, PEARCY & al. 2011). The reproductive systems of *Wasmannia auropunctata*, *Vollenhovia emeryi*, and *Paratrechina longicornis* are

particularly noteworthy. Queens and males are produced asexually in these species, whereas sterile workers are produced sexually (FOURNIER & al. 2005, KOBAYASHI & al. 2008, PEARCY & al. 2011). Clonal production of new queens may be advantageous for social insects because of the high genetic relatedness it achieves within colonies. However, genetic diversity never increases as long as they use only clonal reproduction for production of new reproductives.

In *Vollenhovia emeryi*, most queens do not have the capacity to disperse because of defective short-wings. Our dissection of colonies collected from 2008 to 2010 showed that 98% of short-winged-queens (392 queens from 272 colonies) mate in the nest before hibernation. After hibernation, colonies split by budding (OHKAWARA & al. 2002). Nests of *V. emeryi* are distributed within a small area (see Materials and Methods), and may risk extinction due to a combination of limited dispersal and low effective population size. In this study, we show that colonies produce long-winged queens in addition to short-winged queens. We hypothesize that *V. emeryi* may conditionally use clonal and sexual reproductive systems to produce short- and long-winged queens, respectively, in a manner similar to the facultative sexual / asexual systems of other species (GOUYON 1999, GREEN & FIGUEROLA 2005). To determine the reproductive system of long-winged queens in *V. emeryi*, we used microsatellite markers to compare genotypes of long-winged queens and their parents. In addition, in the laboratory, we tried to identify factors that induce production of long-winged queens by varying nutritional conditions.

## Materials and Methods

**Field collection:** *Vollenhovia emeryi* was identified using the key in THE MYRMECOLOGICAL SOCIETY OF JAPAN (1992); the ant is found in secondary forests throughout Japan. We collected *V. emeryi* colonies at two sites, in Ishikawa (SD site, 36° 35' N, 136° 35' E) and in Toyama Prefectures (TK site, 36° 45' N, 137° 1' E) in Japan, from May to October 2008. Field surveys confirmed that populations at these sites were composed of colonies containing short-winged queens. The SD population occupied an area of about 5 m × 8 m and the TK population was about 3 m × 10 m.

**Microsatellite analysis:** To examine reproductive modes of these colonies, we genotyped all dealate (queens that have lost their wings after mating) queens, the sperm in their spermathecae, all new queens (short- and long-winged queens), and all males from collected colonies containing long-winged queens. In each colony, ten workers were selected randomly and genotyped. DNA extraction and genotyping were performed using methods described previously (OHKAWARA & al. 2006). We amplified DNA microsatellite loci using the following primers: *L-5*, *Myrt-3*, *Vems-14*, and *Vems-78* (KOBAYASHI & al. 2010).

**Conditions for inducing long-winged queens in the laboratory:** Our field observations (see Results) showed that colonies containing more new reproductives produced more long-winged queens, suggesting that nutritionally rich colonies tend to produce long-winged queens in addition to short-winged queens. To determine the effect of nutritional factors on long-winged queen production, we recorded the number of new queens produced in laboratory colonies kept under different conditions, i.e., eight colonies fed once every two days and eight colonies fed once



Fig. 1: Wing dimorphism in *V. emeryi*. (a) Short- and (b) long-winged queens produced from the same colony.

every four days. We balanced the number of workers and queens in each colony between the two experimental groups. These colonies were collected in April 2011 at the SD site and maintained until October 2011. New short- and long-winged queens were dissected to check for insemination by observation of the spermatheca.

## Results

**Morphological differences between short- and long-winged queens:** We collected 63 and 17 colonies from the SD and TK sites, respectively, including new reproductives in 2008. New queens exhibited wing dimorphism in five SD colonies and six TK colonies. These queens were classified according to wing length: short-winged queens (wing =  $1.05 \pm \text{SD } 0.02$  mm,  $n = 10$ , Fig. 1a) and long-winged queens (wing =  $2.96 \pm \text{SD } 0.02$  mm,  $n = 17$ , Fig. 1b). Wing lengths from the two groups differed significantly ( $p < 0.0001$ ,  $F_{1,18} = 1453.32$ , one-way ANOVA).

**Genetic data:** DNA was collected from individuals sampled from 11 colonies (five at site SD and six at TK) that produced both queen morphs. In total, we sampled 38 dealate queens, 95 short-winged queens, 17 long-winged queens, 110 workers, and 51 males. We amplified microsatellite loci *L-5* (7 alleles), *Myrt-3* (3 alleles), *Vems-78* (5 alleles), and *Vems-14* (4 alleles) (Tabs. S1 and S2, Appendix, as digital supplementary material to this article, at the journal's web pages). In five colonies from the SD site, genotypic differences among dealate queens, workers, short-, and long-winged queens, were observed at *L-5*, *Myrt-3*, and *Vems-78*, whereas genotypic differences were observed at *L-5*, *Vems-78*, and *Vems-14* in six colonies from the TK site. All dealate queens and 97.1% and 95% of short winged-queens from the SD and TK sites, respectively, were homozygous for all loci (Fig. 2). In contrast, almost all workers and long-winged queens were heterozygous (Fig. 2).

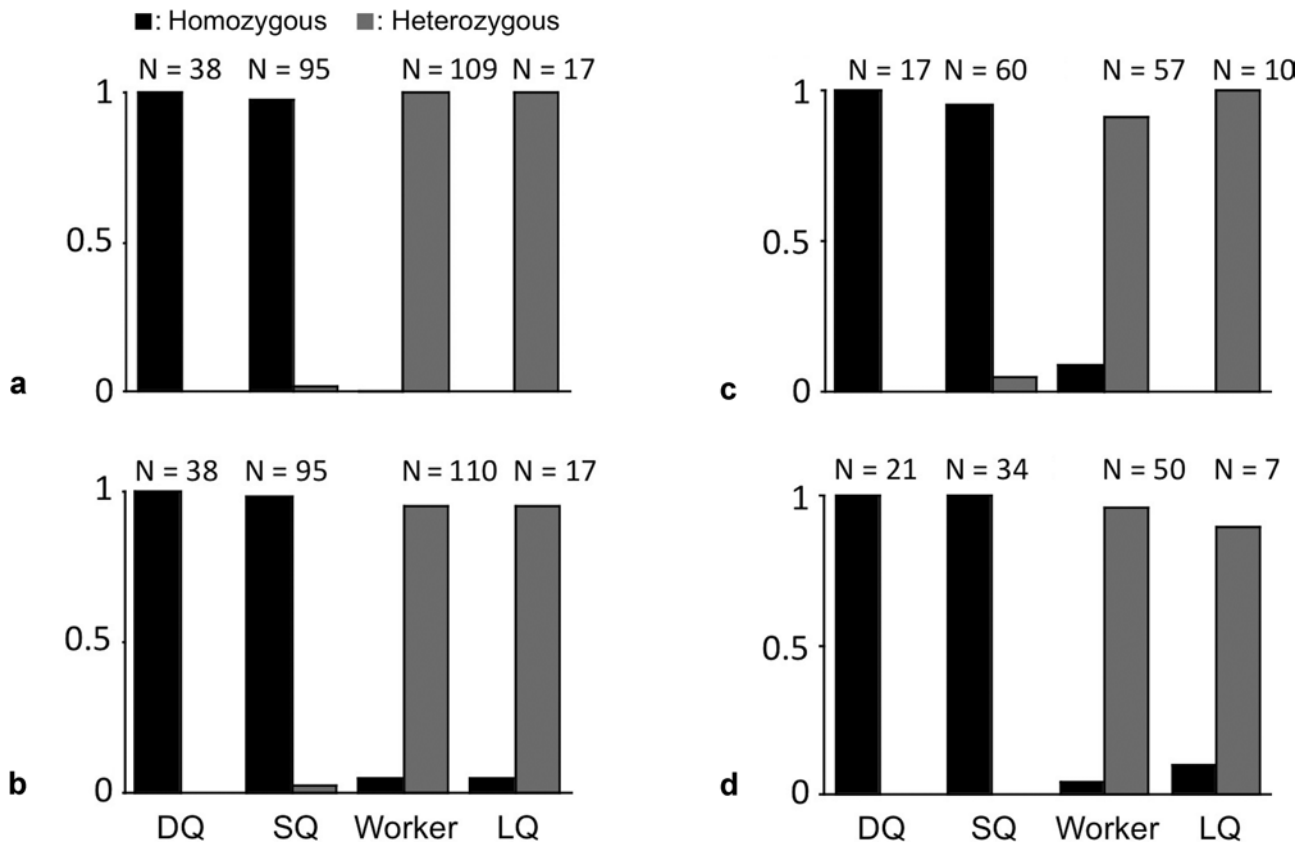


Fig. 2: Frequency of homozygous and heterozygous DQ (dealate queens), SQ (short-winged queens), workers, and LQ (long-winged queens) at four microsatellite loci; (a) *L-5*, (b) *Vems-78*, (c) *Vems-14* and (d) *Myrt-3*.

As shown in Tables S1 and S2, inheritance patterns of genotypes at all microsatellite loci suggest that dealate queens and short-winged queens were produced asexually, whereas workers were produced sexually, as in previous studies of *Vollenhovia emeryi* (OHKAWARA & al. 2006, KOBAYASHI & al. 2008). However, long-winged queens were also heterozygous at most of the same loci as the workers. The heterozygote frequencies of short-, long-winged queens, and workers were different at each locus ( $\chi^2$  test; *L-5*:  $\chi^2 = 250.0$ ,  $P < 0.01$ ; *Vems-78*:  $\chi^2 = 233.5$ ,  $P < 0.01$ ; *Vems-14*:  $\chi^2 = 122.2$ ,  $P < 0.01$ ; *Myrt-3*:  $\chi^2 = 100.9$ ,  $P < 0.01$ ; Fig. 2). Worker and long-winged queens were predominantly heterozygous, whereas almost all short-winged queens were homozygous.

**Conditions for long-winged queen production in the field and laboratory:** For 63 colonies collected at SD and 17 collected at TK, the average number of new queens in colonies producing both short- and long-winged queens ( $31.44 \pm \text{SD } 4.0$ ) was higher than that in colonies producing only short-winged queens ( $8.5 \pm 2.8$ ,  $P < 0.0001$ ,  $Z = 3.97$ ; Mann-Whitney U-test), suggesting that colonies having resources to produce more new reproductives tend to produce long-winged queens in addition to short-winged queens.

Laboratory experiments involved eight colonies. Those supplied with better nutrition produced a total of 20 long-winged queens (average:  $2.5 \pm \text{SD } 3.51$ ) and 37 short-winged queens (average:  $4.6 \pm \text{SD } 11.9$ ), whereas colonies with poorer nutrition did not produce any long-winged queens ( $P < 0.05$ ,  $Z = 2.84$ ; Mann-Whitney U-test).

To check mating behavior of short- and long-winged queens produced in the laboratory, we dissected all 37 short-winged queens and 19 of the 20 long-winged queens (excluding the one female from a colony that produced no males). Dissection data showed that all 37 short-winged queens were inseminated, whereas none of the 19 long-winged queens were.

## Discussion

*Vollenhovia emeryi* employs conditional asexual and sexual production of new queens, which results in short- and long-winged queens, respectively. Such an association between reproductive system and wing morphology has not been reported in any other clonal ants to date. Previously, *V. emeryi* has been characterized as belonging to the group of ants that have strong genetic caste determination (ANDERSON & al. 2008, SCHWANDER & al. 2010). However, colonies that acquired better nutrition not only produced long-winged queens, but also more queens overall, suggesting that environmental factors significantly affect caste determination in this species.

In addition, dissection data suggest that breeding behaviors differ between short- and long-winged queens. In this species, all males have long functional wings and in wild populations they are observed until early summer (OHKAWARA & al. 2002), although most new queens were inseminated during autumn, before hibernation (see Introduction), suggesting that long-winged queens probably mate with males derived from other colonies after hibernation. Some experiments that we performed independently indi-

cated that long-winged queens can mate with males derived from different populations and successfully produce a subsequent generation (M. Okamoto, K. Kobayashi, E. Hasegawa & K. Ohkawara, unpubl.).

Since short-winged queens lack the capacity to disperse, it is difficult for them to invade new habitats. Thus, production of dispersal queens may be advantageous to avoid extinction because of environmental degradation. However, there is no strong evidence regarding reproductive success of long-winged queens. Theoretical studies suggest that even if the survival rate of dispersers is low and the habitats of parents are stable (even if the survival rate is zero), it is still advantageous to produce dispersers (HAMILTON & MAY 1977). Thus, *Vollenhovia emeryi* colonies may also benefit from disperser production for their reproductive success in the long term. Actually, production of long-winged queens in addition to short-winged queens has been observed in other populations in the US (D.B. Booher, unpubl.) and Korea (G. Jeong, unpubl.). In general, sexual reproduction has the potential to rapidly generate novel genetic variation (VAN VALEN 1973, WILLIAMS 1975). Therefore, occasional sexual reproduction of new queens may be advantageous to *V. emeryi*, which has evolved asexual reproduction of both males and females.

In other organisms, dispersing individuals are produced when environmental conditions deteriorate, such as overpopulation, poor nutrition, and declining temperatures (KLEIVEN & al. 1992, APPLEBAUM & HEIFETZ 1999, SIMON & al. 2002). Why then are long-winged queens produced only in colonies with access to good nutrition? In the case of social insects, colonies with few workers or few food resources invest their reproductive resources only in worker production in order to maintain the colonies (SEELEY & MIKHEYEV 2003). Production of new reproductives occurs only in colonies with extra resources (BOURKE & FRANKS 1995, SMITH 2007, TSCHINKEL 2011). Thus, there is a positive correlation between nutrition and production of long-winged queens in *Vollenhovia emeryi*.

As mentioned above, several ant species with clonal reproduction have been reported in recent decades (FOURNIER & al. 2005, KOBAYASHI & al. 2008, PEARCY & al. 2011). Interestingly, these species commonly employ sexual production of workers, suggesting that there are selective advantages to having genetically diverse worker castes, since workers are exposed to various environments (ANDERSON & al. 2008, SCHWANDER & al. 2010, SMITH & al. 2008, SCHMIDT & al. 2011). Our data constitute the first evidence for the conditional use of sexual and asexual reproduction to produce long-winged and short-winged queens, respectively. Finally, we conclude that *Vollenhovia emeryi* has maintained high relatedness within colonies using clonal production for non-dispersing queens, but simultaneously produces a wide array of genotypes by occasional sexual production for dispersing queens, suggesting that they maximize the advantages of clonal and sexual reproduction.

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