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研究課題名(和文) コカミアリに特異的な性決定様式の解明

研究課題名(英文) Study of sex determination mechanisms in *Wasmannia auropunctata*

研究代表者

ミケエヴ アレクサンダー (Mikheyev, Alexander)

沖縄科学技術大学院大学・その他の研究科・准教授

研究者番号：90601162

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研究成果の概要(和文)：アリ・ハチを含む膜翅目昆虫には単一の相補的性決定遺伝子座CSDが存在すると考えられている。ミツバチでは性決定カスケードの最上流に存在するCSDが特定されており、ヘテロ型なら雌、ヘミ・ホモ型なら雄になることが分かっている。本研究では、ウメマツアリを用いて近親交配系を確立し、次世代に生じる二倍体の雄と雌を用いてQTL解析を行い、CSDの存在する領域を調べた。結果、2つの独立な染色体上にCSDの存在が示され、アリ類で初めて相補的性決定遺伝子座の複数化が生じている可能性が示唆された。CSDの複数化は近親交配で生じる不妊個体の出現頻度を従来の半分に抑える長所があり、他の種でも進化している可能性がある。

研究成果の概要(英文)：Recent, though controversial, comparative genomic studies proposed that a core pathway underlies complementary sex determination (CSD) in many, if not all, hymenopteran insects. First characterized in honey bees, it involves the joint action of tandemly arranged homologs of the fruit fly transformer gene. We used an experimental cross to test whether the sex determination mechanism is conserved in the ant *Vollenhovia emeryi*. Two QTLs on separate linkage groups (CsdQTL1 and CsdQTL2) jointly explained 98.0% of the phenotypic variance. CsdQTL1 included two tandem transformer homologs, with one under diversifying selection, as in the honey bee, suggesting a mechanism shared for over 100 million years. CsdQTL2 had no homology to CsdQTL1 and included a region more than 250kb. As multi-locus CSD can collapse to a single gene, alternate loci such as CsdQTL2, can provide intermediates to other sex determination pathways. Multi-locus CSD may explain the diversity of sex determination pathways.

研究分野：生態・環境

キーワード：genomics sex determination reproduction social insects ants

### 1. 研究開始当初の背景

One of the most fundamental choices faced by most organisms during development is whether to become male or female. Mechanisms underlying this determination have downstream implications for a variety of life history traits, such as sex ratios in the population, and even the risk of extinction. For instance, under complementary sex determination (CSD), individuals heterozygous at CSD loci become females, while those homo- or hemizygous become males. CSD systems carry a high penalty for inbreeding, as diploid males resulting from homozygosity at these loci are typically sterile. Low population sizes under CSD can lead populations into an extinction vortex. Consequently, one expects interplay between the molecular mechanisms of sex determination, and the ecology of a particular species. The actual molecular pathways underlying sex determination are diverse, and remain poorly understood. More specifically in insects, although a wide variety of sex determination mechanisms exist, many of them revolve around a core conserved pathway. In the fruit fly (*Drosophila melanogaster*) where sex determination is understood best, this pathway involves the *transformer* (*tra*) gene. Interestingly, there is evidence that *tra* plays a role in sex determination in other species as well. For instance, in honey bees a homolog of this gene has evolved into the *complementary sex determiner* (*csd*) gene locus, acting as a heterodimer to affect the sex-specific

splicing. In honey bees, *csd* arose by duplication of *feminizer* (*fem*), located adjacent to it on the chromosome.

### 2. 研究の目的

There is evidence that *tra* plays a role in a wide variety of hymenopteran sex determination pathways, though this view is disputed. In order to test for involvement of *tra* homologs in hymenopteran CSD pathways, we mapped CSD loci in the ant, *Vollenhovia emeryi*. This ant is particularly suited to linkage mapping analysis because of its unusual reproductive system, which involves parthenogenetic reproduction by queens, and androgenetic reproduction by males, which arise from eggs that lack the queen's genome. Workers are produced sexually, but are sterile, except for some that develop into queens. Sexually produced queens can be crossed with the paternal lineage in the genetic equivalent of a classic backcross.

### 3. 研究の方法

Although we initially proposed to use the little fire ant (*Wasmannia auropunctata*) as the study system, due to delays in getting research permits in Ecuador we switched to a Japanese ant, *V. emeryi* is one of the few ants that can be experimentally crossed in the lab and can be reared across several generations. It provides an excellent model system to address a range of biological questions, including mechanisms of sex determination, genetic caste determination, and social parasitism. We have developed a wide range of genomic resources for this species, including a

genome, transcriptome, linkage map, and population genomic data, using them to investigate the mechanism of sex determination. Using QTL mapping we show that *V. emeryi* has multi-locus CSD (ml-CSD) with two loci, one of them at the same position as duplicated *tra* homologs. We then show that the peculiar reproductive system in this ant serves to eliminate costs of inbreeding, the production of sterile males, by fixing CSD heterozygosity in workers, through fixed differences in male and female clones. Evolution of clonality, in turn, may open novel ecological niches, pre-adapting *V. emeryi* to exist in populations of extremely low genetic diversity and to disperse anthropogenically.

#### 4. 研究成果

##### (1) Genome assembly and annotation.

The genome assembly totaled 287,900,827 bp, including 19,131,583 gaps. It had 13,258 scaffolds (N50 1,346,088) and 23,916 contigs (N50 32,417). As evaluated by BUSCO, the genome assembly was largely complete, with only 16 out of 2675 (0.59%) universal single-copy orthologs missing, and 64 (2.4%) partial genes. The genome annotation contained 14870 coding genes, of which 9239 genes had a known protein with an alignment covering 50% or more of the query, and 2671 had an alignment covering 95%. The complete annotation report is available from NCBI (*Vollenhovia emeryi* Annotation Release 100).

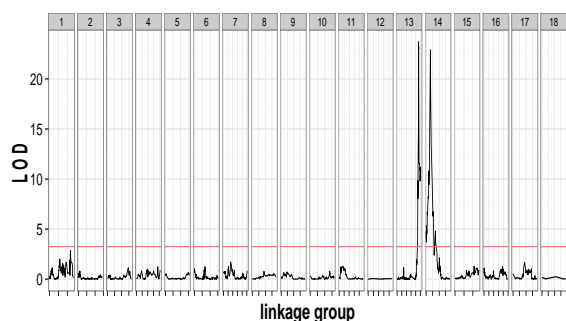
##### (2) Diploid male production from inbred

**and outbred crosses.** A quarter ( $27.1 \pm 8.91\%$  SD) of all offspring in inbred crosses were diploid males, while the rest were workers, and a single queen. By contrast, queens mated to males from other populations (outbred crosses) produced only workers, and a few queens after ten months of laboratory rearing. This ratio is consistent with a two-locus sex determination mechanism.

**(3) Sterility of diploid males.** While seminal vesicles of androgenetic haploid males contained sperm, those of diploid males did not, even five months after eclosion, indicating that diploid males were sterile, and suggesting that inbred queens must invest about 25% of their resources producing reproductively useless males. Correspondingly, inbred queens also produced about 25% fewer workers. Despite the fact that diploid males were immediately removed from the colonies, the number of emerged workers per month produced by inbred queens was significantly lower than for outbred queens ( $8.3 \pm 3.06$  vs.  $13.9 \pm 4.80$ ,  $P < 0.05$ ,  $F_{1,21} = 6.81$ , one-way ANOVA). This suggests that colonies do not sufficiently compensate for the production of diploid males in other ways, such as increasing worker production.

**(3) Linkage map and QTL analysis.** The cross contained 239 individuals genotyped at 3541 markers with 1.9% missing data, and a genotype error rate of 0.26% inferred from quality scores. These markers clustered into 18 linkage groups, consistent with previous

karyotype estimates<sup>5</sup>. A genome scan identified two QTLs of large effect (*CsdQTL1* and *CsdQTL2*), located on linkage groups 13 and 14, which jointly accounted for almost all of the phenotypic variance in diploid male production (98.0%). The two regions can be explored interactively using the NCBI genome browser (*CsdQTL1*: <http://goo.gl/oNp5Jq>, *CsdQTL2*: <http://goo.gl/8x3MLE>). Located on LG14, *CsdQTL1* includes three tightly linked (0 cM apart) markers spanning 38kb and located 20kb from two tandem *tra* homologs. This arrangement is typical of other ants. *CsdQTL2* spans 3.2 cM and covers a range of at least 236kb on scaffold NW\_011967112.1, which is spanned by eight markers, co-located to two positions. It also includes one marker from scaffold NW\_011967235.1, which probably results from a scaffolding error, since the rest of that scaffold is on LG2. Although QTL analysis is agnostic about the effect of heterozygosity on phenotype, diploid male phenotypes are associated with homozygosity at these loci, consistent with the proposed mechanism of sex determination. There was no homology between *CsdQTL1* and *CsdQTL2* suggesting that these loci did not emerge by duplication.



**Linkage mapping confirms the existence of two-locus CSD in *V. emeryi*. Marker locations are shown by short dashes along the x-axis of the plot. A red line shows the permuted 95% significance level of the LOD score. This genome scan found two loci, named *CsdQTL1* and *CsdQTL2*, on LG13 and LG14, respectively. These two loci and their interaction explained 98.0% of the observed phenotypic variance.**

QTL mapping analysis shows that *V. emeryi* has two unlinked sex determination loci (*CsdQTL1* and *CsdQTL2*). The *CsdQTL1* locus has duplicated *tra* homologs, as is typical of ants. This configuration resembles the sex determination locus of honey bees, where *csd* is located adjacent to *fem*, with both genes acting in the sex determination pathway. As ant and bees have diverged more than 100 million years ago, this suggests that sex determination honey bees and *V. emeryi* is homologous, forming the core of an ancient hymenopteran pathway. Alternatively, CSD could have evolved separately in both lineages by convergent co-option and duplication of *fem* for CSD. The latter scenario seems less likely, given the frequent co-occurrence of syntenic *tra* homologs across a range of hymenoptera.

5. 主な発表論文等  
(研究代表者、研究分担者及び連携研究者には下線)

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## 6. 研究組織

### (1) 研究代表者

ミケエエブ アレクサンダー

(Mikheyev, Alexander)

沖縄科学技術大学院大学・

生態・進化学ユニット・准教授

研究者番号：90601162