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Colony social structure in native and invasive populations of the social wasp *Vespula pensylvanica*

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Abstract Social insects rank among the most invasive of terrestrial species. The success of invasive social insects stems, in part, from the flexibility derived from their social behaviors. We used genetic markers to investigate if the social system of the invasive wasp, *Vespula pensylvanica*, differed in its introduced and native habitats in order to better understand variation in social phenotype in invasive social species. We found that (1) nestmate workers showed lower levels of relatedness in introduced populations than native populations, (2) introduced colonies contained workers produced by multiple queens whereas native colonies contained workers produced by only a single queen, (3) queen mate number did not differ significantly between introduced and native colonies, and (4) workers from introduced colonies were frequently produced by queens that originated from foreign nests. Thus, overall, native and introduced colonies differed substantially in social phenotype because introduced colonies more frequently contained workers produced by multiple, foreign queens. In addition, the similarity in levels of genetic variation in introduced and native habitats, as well as observed variation in colony social phenotype in native populations, suggest that colony structure in invasive populations may be partially associated with social plasticity. Overall, the differences in social structure observed in invasive *V. pensylvanica* parallel those in other, distantly related invasive social insects, suggesting that insect societies often develop similar social phenotypes upon introduction into new habitats.

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Introduction

Social insects rank among the most widespread and damaging of invasive organisms (Moller 1996; Rust and Su 2012; Suarez et al. 2008). A diversity of introduced termites, ants, bees, and wasps have produced substantial ecological and economic damage in their invaded habitats (Beggs et al. 2011; Holway et al. 2002; Kenis et al. 2009; Lowe et al. 2000; Vargo and Husseneder 2009; Williams 1994). The tremendous success of invasive social insects stems partly from their dynamic social systems (Suarez et al. 2008). The cooperative societies displayed by social insects provide great flexibility and readily change to allow invading social insects to operate efficiently in new habitats (Moller 1996). Consequently, the social systems of many invasive social insects differ in their native and introduced ranges (Beggs et al. 2011; Holway et al. 2002; Leniaud et al. 2009; Silverman and Brightwell 2008; Vargo and Husseneder 2009).

Vespula social wasps, commonly known as yellowjackets in North America, represent important taxa for studying and understanding social insect invasions. *Vespula* wasps are native and abundant in many parts of the Northern Hemisphere (*Vespula* distribution and natural history reviewed by Akre and MacDonald 1986; Edwards 1980; Greene 1991; Jeanne 1980; Spradbery 1973). In their native ranges, *Vespula* species usually display an annual life cycle.

The annual *Vespula* life cycle begins when a single multiply-mated (polyandrous) queen initiates a nest in the spring. The queen produces workers during the spring and summer months. Once the workers mature, they take over the task of colony maintenance. However, the queen remains wholly responsible for the production of all offspring within the nest as long as she is present; thus an annual colony only contains one, functional queen (i.e., is monogyne). In early autumn, the colony begins to produce males and new reproductive queens. These queens and males partake in mating flights. The mated queens find a safe place to overwinter. The remainder of the population, including all males, workers, and old queens, do not survive the winter. Thus, most *Vespula* colonies survive only a single year in their native ranges.

Several *Vespula* species have been introduced into new habitats (reviewed by Beggs et al. 2011). These introduced *Vespula* cause severe ecological problems in their new ranges (Beggs 2001; Beggs et al. 2011; Hanna et al. 2013). In addition, *Vespula* colonies in some of these introduced regions, including New Zealand (Gambino 1991), Australia (Spradbery 1973), South America (Jeanne 1980), Hawaii (Gambino et al. 1990; Wilson et al. 2009), and Africa (Akre and Reed 1981) can survive for multiple years.

This study focuses on the western yellowjacket, *Vespula pensylvanica*. *V. pensylvanica* is native to the western half of the temperate regions of North America (Akre et al. 1980). However, *V. pensylvanica* was accidentally introduced into the Pacific islands of Hawaii. *V. pensylvanica* was first documented on the island of Kauai in 1919. It has subsequently invaded six of the eight main Hawaiian Islands (Gambino et al. 1990). Like other introduced *Vespula* species, *V. pensylvanica* sometimes forms large, perennial nests in its introduced range (Akre and Reed 1981; Gambino 1991; Gambino et al. 1990; Ratnieks et al. 1996; Reed and Landolt 2005; Wilson et al. 2009).

The primary aim of this research was to investigate if the social structure of *V. pensylvanica* colonies differed in native and introduced habitats. In addition, we were interested in determining if different social insect species tended to show similar social structures in invasive ranges. We found evidence of substantial differences in colony genetic structure between native and invasive *V. pensylvanica*. Native colonies were always headed by a single queen, whereas invasive colonies were frequently headed by multiple, unrelated queens. In addition, the patterns we observed paralleled those documented in other introduced social insects, suggesting that the social systems of diverse insects change in similar ways in novel habitats.

Materials and methods

We collected *V. pensylvanica* workers from 18 colonies in a native population near Olympia, WA, USA and 32 colonies in an introduced population in Hawaii Volcano National Park, HI, USA between August and October of 2008 and September and October of 2010 (Table 1). The maximum distance between nests in Washington and Hawaii was 48.8 and 14.7 km, respectively. Collected specimens were

Table 1 Characteristics and collection locations for *V. pensylvanica* colonies sampled from a native population in Washington and an introduced population in Hawaii

Population	Colony	Latitude	Longitude	Gyny	k	k_{e3}
Washington	G192	47.023	−122.884	M	7	5.30
	G1822	47.030	−122.789	M	3	2.15
	G4822	47.005	−122.751	M	6	1.57
	G5931/0	47.000	−122.800	M	6	4.90
	G61010	47.000	−122.918	M	2	1.13
	G8826	46.829	−123.049	M	3	1.77
	J193	–	–	M	2	1.74
	J294	47.266	−123.100	M	7	5.98
	J393	47.070	−122.820	M	5	4.00
	J497	46.990	−122.811	M	4	3.97
	J593	–	–	M	6	3.38
	J693	47.084	−122.896	M	5	5.20
	J818	47.039	−122.766	M	4	2.19
	J1081	47.012	−122.811	M	3	1.78
	J1101	46.907	−122.906	M	3	1.81
	J1928	46.964	−122.958	M	3	2.48
	J4929	47.058	−122.824	M	3	2.14
	J7830	47.007	−122.862	M	6	3.84
Hawaii	ARL1	19.340	−155.224	P		
	ARL2	19.341	−155.223	M	4	1.86
	ARL3	19.340	−155.224	P		
	ARL4	19.340	−155.224	M	5	7.23
	ARL5	19.340	−155.224	P		
	ARL6	19.340	−155.224	P		
	AT07	–	–	ND		
	CR2	19.417	−155.250	P		
	CR3	19.417	−155.251	P		
	CR4	19.418	−155.253	P		
	CR5	19.419	−155.253	P		
	CRT1	–	–	P		
	CRT10	–	–	P		
	EW2	19.372	−155.242	P		
	EW3	19.357	−155.253	P		
	EW4	19.308	−155.304	P		
	EW5	19.356	−155.254	P		
	EW7	19.349	−155.261	P		
	EW8	19.352	−155.259	P		
	HP5	–	–	ND		
	KD1	19.339	−155.269	P		
	LKK1	19.337	−155.213	M	10	14.92
	MU1	19.365	−155.218	M	4	3.07
	MU2	19.365	−155.215	P		
	MU3	19.365	−155.215	P		
	MU4	19.364	−155.217	ND		
	MU20	19.366	−155.223	P		
	PA2	19.371	−155.226	P		
	PJ1	19.366	−155.247	P		

Table 1 continued

Population	Colony	Latitude	Longitude	Gyny	k	k_{e3}
	RS1	19.420	−155.251	P		
	VS1	19.425	−155.240	P		
	WL2	19.425	−155.257	M	4	3.15

Colonies were deemed to be monogyne (M) or polygyne (P) based on genotypes of workers. Minimum mate number (k) and effective mate number (k_{e3}) of queens heading monogyne colonies are provided

ND = no data are provided because colonies were not assayed at nuclear loci. – = Exact location of colony not measured

immediately frozen or placed in 95 % ethanol for subsequent DNA analysis.

DNA was extracted from single legs of *V. pensylvanica* workers using a variation of the Chelex protocol (Goodisman et al. 2001a). The single leg preparations provided sufficient DNA for PCR amplification of microsatellite loci. We used agarose gel electrophoresis as described by Hoffman et al. (2008) to determine if 43 microsatellite loci developed in other vespine wasps provided useful genetic information in *V. pensylvanica* (Table 3, "Appendix"). We ultimately obtained the multilocus genotype of 954 V. *pensylvanica* workers at the 10 microsatellite loci LIST2004, LIST2007, LIST2008, LIST2010, LIST2014, LIST2015, LIST 2020, Rufa 5, Rufa 19, and VMA 6 (Daly et al. 2002; Hasegawa et al. 2002; Thoren et al. 1995).

Allele frequencies within the Washington and Hawaiian populations were calculated using the program RELATEDNESS 5.0.8 weighting colonies equally (Queller and Goodnight 1989). The number of alleles and expected heterozygosities were determined separately for the Washington and Hawaiian populations. We used a paired *t* test to investigate if the expected heterozygosities in Hawaii and Washington differed significantly.

We tested for genotypic disequilibrium between microsatellite markers using the program GENEPOP (Raymond and Rousset 1995). To avoid potential problems caused by nonindependence of worker genotypes from the same colony, we randomly selected one individual worker genotype per colony to create a new data set with a number of individuals equal to the total number of colonies. This procedure was repeated 10 times to produce 10 such datasets. Each data set was used to calculate the levels of linkage disequilibrium between pairs of loci, and the median of the 10 values was taken as an estimate unbiased by colony structure.

We used the program MATESOFT 1.0 to determine if workers from each colony could have been produced by a single queen and to calculate the minimum number of male mates needed to explain the genotypes of workers in putative monogyne colonies (Moilanen et al. 2004). MATESOFT also provided the expected genotype of the queen and her mates in monogyne colonies. When more than one combination of putative parental genotypes could explain the observed worker genotypes, hypothetical parental genotypes were selected according to the following rules: (1) the queen genotype that would result in the fewest separate patrilines was chosen, (2) if more than one queen genotype yielded the same minimal number of patrilines, the queen with the most probable genotype was selected, and (3) within each set of potential male mates, the most probable genotype was selected. We used ANOVA to determine if the minimum number of male mates of queens from putatively monogyne colonies differed between the Hawaiian and Washington populations.

We calculated the effective paternity (k_{e3}) of queens heading monogyne colonies (Nielsen et al. 2003) to take into account the number of times a queen mated and the unequal contributions of her mates to her offspring. Effective paternity measures the amount of skew among males mated to single queens within colonies; k_{e3} is expected to be low in colonies where few males dominate reproduction and high in colonies where all males contribute equally to reproduction. Workers that could not be unambiguously assigned to one patriline were left out of the calculation. We used ANOVA to determine if k_{e3} differed for queens in the Hawaiian and Washington populations.

The relatedness of nestmate workers was estimated using RELATEDNESS 5.0.8. We also estimated the relatedness of queens to their putative male mates in monogyne colonies. Potential differences in allele frequencies between Hawaii and Washington were taken into account by using the "deme" function. Colonies were weighted equally and standard errors for estimates were obtained by jackknifing over colonies. We used ANOVA to determine if relatedness estimated differed for nestmates in Washington and Hawaii.

We investigated the distribution of mitochondrial DNA (mtDNA) haplotypes in workers sampled from Hawaiian colonies. For mtDNA analyses, DNA was extracted from thoraces or gasters of *V. pensylvanica*

workers using either the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) or the EZNA Tissue DNA kit (Omega Bio-tek, Norcross, GA, USA). We tested combinations of 24 primers previously developed to PCR-amplify mtDNA in other insect taxa for their ability to amplify mtDNA fragments in *V. pensylvanica* (Carew et al. 1997; Chiotis et al. 2000; Foitzik and Herbers 2001; Gadau et al. 1999; Hasegawa et al. 2002; Ross and Shoemaker 1997; Simon et al. 1994; Tay et al. 1997; Wetterer et al. 1998). Of the 27 pairs tested, 12 successfully amplified in *V. pensylvanica* (Table 4, "Appendix").

We sequenced amplicons obtained from the primer pairs Ben/Jerry, CB3ext/tRs2, CI13/CI14, and L3034/H3665 from 24 workers from 13 different Hawaiian nests. We uncovered a total of 12 polymorphic nucleotides that distinguished three haplotypes in this population (GenBank JQ235071–JQ235164). We found that restriction digestion of the CI13/CI14 amplicon using MseI (New England Biolabs, Ipswich, MA, USA) produced banding patterns that were sufficient to distinguish between these three haplotypes using agarose gel electrophoresis. We subsequently used this MseI RFLP assay to determine the mitochondrial haplotype of 278 workers from the Hawaiian colonies. Washington samples were not assayed because nuclear genotypes of workers indicated that all Washington colonies were monogyne (see below).

Results

We obtained the nuclear genotypes of 25.00 ± 13.45 ($\bar{x} \pm$ SD) *V. pensylvanica* workers from 18 Washington colonies and 15.75 ± 9.80 workers from 27 Hawaiian colonies (Table 1). We found that the ten microsatellite loci used for the study possessed many alleles and relatively high expected heterozygosities in both populations, thereby providing the variation necessary to determine the social structure of colonies (Table 2). For instance, the probability of two *V. pensylvanica* males having the same multilocus genotype in either Washington or Hawaii was $\ll 0.0001$. In addition, the expected heterozygosities of loci in Hawaii were marginally lower than those in Washington (paired t test, $t_9 = -2.19$, $P = 0.056$). We also found no evidence for genotypic linkage disequilibrium between any pairs of nuclear loci

Table 2 Levels of genetic variation in a native Washington (WA) and introduced Hawaiian (HI) population of *V. pensylvanica*
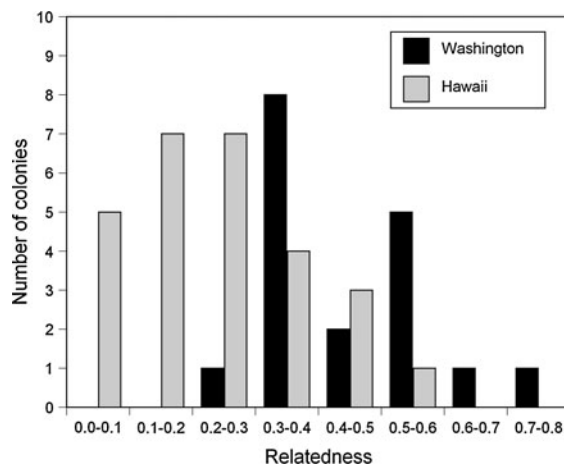
Locus	Alleles		H_e	
	WA	HI	WA	HI
LIST2004	9	8	0.79	0.77
LIST2007	12	11	0.88	0.82
LIST2008	6	5	0.75	0.70
LIST2010	12	8	0.84	0.78
LIST2014	12	11	0.81	0.71
LIST2015	7	9	0.78	0.81
LIST2020	17	13	0.91	0.82
RUFA5	12	7	0.85	0.69
RUFA19	13	9	0.76	0.84
VMA6	19	14	0.90	0.85
mtDNA	ND	3	ND	0.62

Alleles = number of alleles, H_e = expected heterozygosity. For the mitochondrial marker, alleles should be interpreted as haplotypes and expected heterozygosity as gene diversity. ND = no data provided because Washington samples were not assayed at the mitochondrial marker

Fig. 1 Distribution of relatedness values for nestmate *V. pensylvanica* workers collected in native Washington and introduced Hawaiian populations

($P > 0.35$ for all pairs) thereby indicating that the genetic loci would provide independent information.

We investigated if the genotypes of workers within colonies were consistent with the colony being headed by a single queen. We found that workers from all 18 of the Washington colonies in the native population exhibited nuclear genotypes consistent with having been produced by a single polyandrous queen (Table 1). However, worker genotypes from only five of the 27 Hawaiian colonies could be explained as having been produced by a single queen; the workers from the other Hawaiian colonies must have been produced by multiple queens (Table 1).

We found that the mean number of male mates of queens in Washington (4.33 ± 0.45, $\bar{x} \pm$ SEM) did not differ significantly from the mean number of mates of monogyne queens in Hawaii (5.40 ± 0.85; $F_{1, 21} = 1.24$, $P = 0.277$). The mean effective paternity (k_{e3}) of queens in Washington (3.07 ± 0.64) was lower than the mean number of mates (Table 1). In contrast the mean effective paternity in Hawaii (6.04 ± 1.21) was slightly higher than the mean monogyne mate number. This suggests that sampling from these Hawaiian colonies may not have been sufficient to fully determine patriline number (Nielsen et al. 2003). Moreover, the mean effective paternities

of queens in putatively monogyne colonies in the two populations showed marginally significant differences ($F_{1, 21} = 4.71$, $P = 0.042$).

We next investigated if the relatedness of worker nestmates in Washington and Hawaii differed from each other. We found that the relatedness of nestmate workers in Washington (0.44 ± 0.03) and Hawaii (0.24 ± 0.02) differed significantly ($F_{1, 43} = 29.34$, $P < 0.0001$; Fig. 1). We also found that the mean relatedness of inferred male genotypes to inferred queen genotypes in Washington (0.02 ± 0.06) and Hawaii (0.09 ± 0.11) did not differ from zero, as judged by the fact that the 95 % confidence intervals of both estimates overlapped zero. Moreover, the estimates of relatedness of males to queens did not differ significantly between the two populations ($F_{1, 21} = 0.62$, $P = 0.258$).

We determined the mtDNA haplotype from 8.68 ± 5.16 workers ($\bar{x} \pm$ SD) from 31 of the Hawaiian colonies. Our analyses of mtDNA within the Hawaiian population uncovered variation that corresponded to the presence of three distinct haplotypes (haplotypes A, B, and C). Workers from seven of the 31 assayed Hawaiian colonies contained workers of multiple haplotypes (Fig. 2).

Discussion

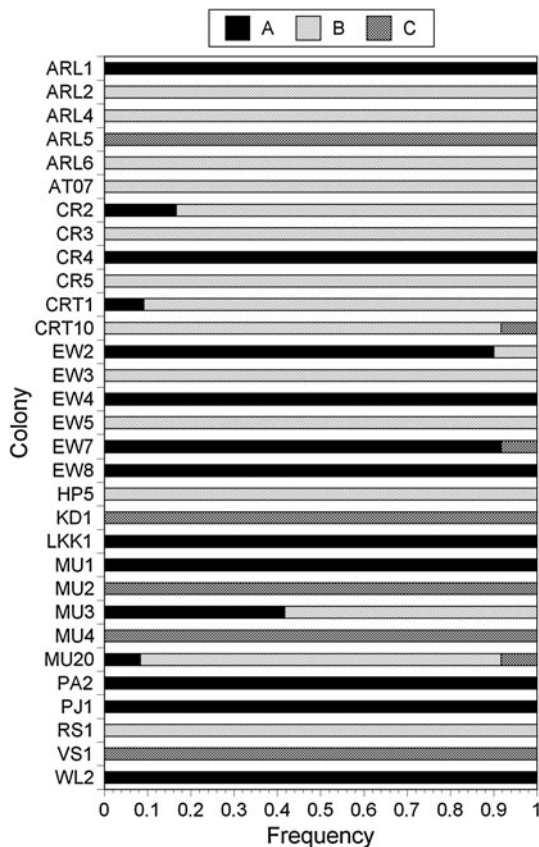The goals of this study were to investigate the social structure of *V. pensylvanica* colonies in native and

Fig. 2 Frequency of three mitochondrial DNA haplotypes (*A*, *B*, and *C*) in workers from 31 *V. pensylvanica* colonies collected from their introduced habitat in Hawaii

introduced habitats, and determine if differences in social structure paralleled those observed in other social insects. We found that *V. pensylvanica* colonies from the native and introduced populations differed in queen number, relatedness, and nestmate origin. Thus, native and introduced *V. pensylvanica* show substantial differences in colony social structure. Moreover, these differences parallel those seen in other introduced social insects suggesting development of similar social phenotype among invasive social insect taxa.

Invasive and native colonies differ in colony queen number

We found that *V. pensylvanica* colonies sampled from our native population were always headed by a single queen. This is typical for *Vespula* wasps in most of their native habitats (Foster and Ratnieks 2001;

Goodisman et al. 2002; Hoffman et al. 2008; Wenseleers and Ratnieks 2006). In contrast, invasive *V. pensylvanica* colonies almost always contained workers produced by multiple females. In addition, multiple queens were discovered inhabiting several of the Hawaiian nests. Thus we infer that *V. pensylvanica* colonies are often headed by multiple reproductive females at the same time (i.e., are polygyne) in their introduced range.

The observation that introduced *V. pensylvanica* colonies are polygyne is consistent with prior information on invasive *Vespula*. Perennial *Vespula* colonies have been found to contain multiple laying reproductives and can survive for more than a single season (Akre and Reed 1981; Gambino 1991; Goodisman et al. 2001b; Greene 1991; Ratnieks et al. 1996; Reed and Landolt 2005; Ross and Visscher 1983; Visscher and Vetter 2003). This variation in colony queen number represents an important change in social structure (Bourke and Franks 1995; Keller 1993).

Polygyne vespine colonies could arise either because new queens are recruited from within their natal nests or because foreign queens enter already established nests (Archer 2010; Keller 1995). A method of differentiating these two models is to examine maternally-inherited genetic markers, such as those found in mitochondrial DNA (Goodisman and Ross 1998). If colonies are initiated by a single queen, and additional queens are subsequently recruited from their natal nest, then nestmates should always possess one mitochondrial DNA haplotype (i.e., that of the original mother queen). However, if foreign individuals enter nests, then individuals from single colonies may contain multiple mitochondrial haplotypes.

Remarkably, we found that workers from introduced *V. pensylvanica* colonies frequently displayed multiple mitochondrial haplotypes as a group. This indicated that the workers belonged to multiple distinct matrilines and, therefore, could not have been produced by a single queen or multiple queens that were produced by a single queen. The presence of multiple mitochondrial haplotypes among workers could result from three mechanisms. First, two queens with different mitochondrial haplotypes could co-found a nest together. However, this explanation is inconsistent with the known biology of *Vespula* (Greene 1991). Second, unrelated workers could join an established colony. This type of worker drift is known to occur in some

social bees and wasps (Beekman and Oldroyd 2008; Sumner et al. 2007; Uddin and Tsuchida 2012; Ulrich et al. 2009). Finally, unrelated queens could join an established nest to reproduce. The previous discovery of multiple queens in *Vespula* nests with no queen cells, indicating that no queens were produced in the focal nests, provides support for this third explanation in this species (Gambino 1991; Ross and Matthews 1982; Spradbery 1991).

In general, polygyny arising from the recruitment of unrelated individuals is somewhat unexpected because addition of nonnestmate queens will lower relatedness among interacting individuals, thus potentially decreasing inclusive fitness benefits (Bourke and Franks 1995; Crozier and Pamilo 1996). Over the long term, such a system may be evolutionarily unstable (Bourke and Franks 1995; Helantera et al. 2009; Keller 1993; Queller and Strassmann 1998). Consequently, the presence of multiple matrilines within *V. pensyl-vanica* colonies indicates a breakdown in colony boundaries and a significant change from typical colony social structure.

The differences in native and introduced *V. pensyl-vanica* social structure may result from plasticity triggered by environmental variation or evolutionary (i.e., genetic) changes. Environmental conditions have been suggested as being important factors in affecting the plasticity of colony social structure (Bourke and Franks 1995; Gambino 1991; Herbers and Banschbach 1999; Hölldobler and Wilson 1977; Keller 1993; Ross and Visscher 1983; Strassmann and Queller 1989; Vetter and Visscher 1997; Visscher and Vetter 2003). However, genetic mechanisms have also been proposed as explanations for the acceptance of unrelated queens within introduced social insect colonies (Giraud et al. 2002; Suarez et al. 2008; Tsutsui et al. 2003).

Our investigation provides some evidence that phenotypic plasticity may be involved in differences in *V. pensylvanica* social structure. For example, although *V. pensylvanica* displays an annual life cycle throughout the majority of its native range, it occasionally shows social structure in the warmer regions of its native range similar to that observed in its introduced range in Hawaii. Specifically, *V. pensyl-vanica* queens in native habitats have been found to overwinter in their natal nest (Vetter and Visscher 1997) and, rarely, form polygyne or perennial colonies

(Ratnieks et al. 1996; Vetter and Visscher 1997; Visscher and Vetter 2003). Thus the observed increase in colony queen number in invasive colonies may represent a shift in frequency of the polygyne pheno-type arising from social plasticity linked to novel environmental conditions. Indeed, environmental var-iation, such as climate and resource abundance, has been found to influence the duration of queen diapause (Ross and Visscher 1983), the use of natal nests by queens for overwintering (Visscher and Vetter 2003), colony queen number (Spradbery 1973), and the longevity of colonies (Ross and Visscher 1983) in *Vespula* species.

Alternatively, the differences between the social systems in native and introduced *V. pensylvanica* populations may have a genetic basis. For example, loss of genetic variation in introduced populations has been hypothesized to account for differences in social phenotype in some invasive social insects. Specifi-cally, it has been suggested that loss of genetic variation at putative recognition loci could lead to a breakdown of colony boundaries associated with the formation of polygyne colonies (Helantera et al. 2009; Suarez et al. 2008).

We found that native and invasive populations of *V. pensylvanica* showed similar, albeit marginally statistically different, levels of genetic variation at our assayed microsatellite loci. The similarity in expected heterozygosities is consistent with the idea that the introduced populations have not undergone substantial evolution (i.e., genetic change) as a result of severe bottlenecks (Table 2). However, considerably more data will be needed to determine if native and introduced populations possess different levels of genetic variation throughout their range. Moreover, determining if genetic changes have specifically affected genes involved in recognition would require documentation of genetic variation at these unknown loci. Overall, the relative importance of genetic change in affecting colony queen number and colony boundaries in introduced social insect colonies remains controversial (Chapman and Bourke 2001; Pedersen et al. 2006; Suarez et al. 2008; van der Hammen et al. 2002; Vasquez and Silverman 2008), because it is difficult to firmly establish if genetic differences cause observed variation in social behaviors.

Queens from invasive and native populations do not differ in mate number

We found that *V. pensylvanica* queens mated with multiple males in both their introduced and native populations. All known *Vespula* species are polyandrous (Foster and Ratnieks 2001; Goodisman et al. 2002; Hoffman et al. 2008; Strassmann 2001), thus our finding was not unexpected. In general, polyandry is predicted to be costly to females because it may result in wasted energy output, an increased risk of predation, and a higher probability of contracting disease (Arnqvist and Nilsson 2000; Jennions and Petrie 2000; Zeh and Zeh 2001). In addition, polyandry in social insects alters the genetic structure of colonies and decreases group relatedness (Crozier and Fjerdingstad 2001).

Several explanations have been put forward to explain the possible benefits of polyandry in social insects. For example, queens may mate with multiple males to reduce the risk of sperm depletion (Bekkevold et al. 1999; Cole 1983; Fjerdingstad and Boomsma 1998). Alternatively the increased genetic variation may lead to increased disease resistance or other genetic benefits (Arnqvist and Nilsson 2000; Brown and Schmid-Hempel 2003; Crozier and Fjerdingstad 2001; Schmid-Hempel 1994). The possible benefits of multiple mating in vespine wasps remain unclear. However, *V. maculifrons* colonies show variation in sex ratio in response to variation in queen mate number (Goodisman et al. 2007; Johnson et al. 2009) suggesting that polyandry influences important aspects of *Vespula* colony function.

Our study was primarily aimed at determining if invasive and native population of *V. pensylvanica* showed changes in social biology. We thus investigated if queen mate number differed between introduced and native populations. Differences in queen mate number may arise if ecological factors affecting mating success, such as nest density or predator behavior, differ between populations.

We found that queen mate number did not differ significantly between introduced and native populations. This result, however, must be viewed cautiously as our power to detect differences in queen mate number was relatively low. Regardless, few studies have investigated if mate number varies in different populations of polyandrous social insect species (Goodisman et al. 2002). This represents a gap in our understanding of the breeding system of social insects.

Changes in social system and the success of invasive social insects

Examination of differences in the social structure of *V. pensylvanica* in native and introduced ranges provides some insight into the mechanisms that allow social insect invaders to become successful (Ross et al. 1996). In Hawaii, the milder climate may lead to the relaxation of climate related constraints (e.g., prey availability), which may affect the physiology of *V. pensylvanica* queens (Ross and Visscher 1983) and colony longevity (Gambino 1991; Wilson et al. 2009). Newly emerged queens may thus enter established nests to immediately initiate oviposition, rather than attempting to hibernate.

Under these conditions, introduced perennial, polygyne colonies may become very large and take in a great biomass and diversity of prey items (Wilson et al. 2009). The formation of perennial colonies may thus increase colony reproductive output. The reduced fitness returns for workers and increased competition among breeders within introduced polygyne colonies may be offset by the longevity and numerical dominance resulting from the plasticity in social organization, at least in the short term (Bargum and Sundstrom 2007; Keller 1995; Wilson 1971).

Polygyny is a likely prerequisite (Gambino et al. 1990; Ross and Visscher 1983), and an established characteristic, of successful perennial colonies (Goodisman et al. 2001b). Polygyne perennial colonies have severe ecological impacts locally because they are dramatically larger than annual colonies and bestow continual predation and competitive pressure on native ecosystems (Beggs 2001; Beggs et al. 2011; Hanna et al. 2012, 2013; Wilson and Holway 2010; Wilson et al. 2009). Consequently, the dynamic and facultative nature of the social behavior of *Vespula* appears to be a critical factor contributing to their success as invasive species.

Parallels in social structure of invasive social insects

The change in the social structure of invasive *V. pensylvanica* offers an important parallel to changes in social dynamics observed in other invasive social insects. In particular, many previous studies of invasive ants (reviewed by Helantera et al. 2009) and sometimes termites (Goodisman and Crozier 2002;

Leniaud et al. 2009) have documented increases in colony queen number, decreases in the strength of colony boundaries, and decreases in nestmate relatedness in invasive or disturbed (i.e. "novel") habitats relative to native habitats.

The changes in social structure observed in introduced *V. pensylvanica* in Hawaii are not as dramatic as those seen in many invasive ants (Helantera et al. 2009). However, the overall patterns are similar. Consequently, these social phenotypes appear to be generally applicable to, and an important component of, successful social insect invasion. In addition, the parallel change in the social structure of these invasive social insect taxa represents a major evolutionary curiosity because individuals often cooperate with unrelated individuals. Further elucidating the relative importance of phenotypic plasticity or adaptive evolution in producing this shift in social structure will contribute to our understanding of social evolution, invasiveness, and the association between the two.

Appendix

See Tables 3 and 4.

Table 3 Approximate size of amplicons and annealing temperatures (T_a) for microsatellite loci that amplified in *V. pensylvanica*

Locus	Amplify[a]	T_a
LIST2001	0	–
LIST2002	V	55
LIST2003	V	55
LIST2004	V	55

Table 3 continued

Locus	Amplify[a]	T_a
LIST2006	+	55
LIST2007	V	58
LIST2008	V	58
LIST2009	+	55
LIST2010	V	65
LIST2011	+	50
LIST2012	0	–
LIST2013	V	50
LIST2014	V	60
LIST2015	V	55
LIST2016	+	55
LIST2017	V	55
LIST2018	0	–
LIST2019	+	60
LIST2020	V	55
Rufa1	0	–
Rufa2	0	–
Rufa3	V	60
Rufa4	0	–
Rufa5	V	55
Rufa6	0	–
Rufa7	+	52
Rufa8	0	–
Rufa9	V	55
Rufa10	+	45
Rufa11	+	55
Rufa12	+	52
Rufa13	0	–
Rufa14	+	55
Rufa15	0	–
Rufa16	0	–
Rufa17	+	58
Rufa18	0	–
Rufa19	V	58
VMA 3	V	55
VMA 4	0	–
VMA 6	V	55
VMA 7	+	68
VMA 8	V	55

[a] Locus failed to PCR-amplify (0), PCR-amplified but was not variable (+), or both PCR-amplified and was variable (V)

Table 4 Approximate size of amplicon and annealing temperature (T_a) for mitochondrial DNA loci that amplified in *V. pensylvanica*

Primer1	Primer2	Amplify[a]	T_a
Barbara	CI13	0	–
Ben	Jerry	+	46
CB1	CB2	+	46
CB1	tRs2	0	–
CB3ext	tRs2	+	46
CB3ext	ND1	0	–
CB7	tRs2	0	–
CI13	CI14	+	46
CI13	Ben	0	–
CI13	H3389	0	–
CI13	H3665	0	–
CI21	CI24	0	–
CO1-RCR	Barbara	0	–
George	Marilyn	+	46
George	H3389	+	46
George	Barbara	0	–
George	H3665	0	–
J2791	H3389	+	46
J2791	H3665	0	–
J2791	Marilyn	+	46
Jerry	H3665	+	46
Jerry	Marilyn	0	–
Jerry	H3389	0	–
L3034	H3389	+	46
L3034	H3665	+	55
ND1	CB1	0	–
ND1	ND4	+	46

[a] Locus failed to PCR-amplify (0) or PCR-amplified (+)

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