

Effects of Size, Shape, Genotype, and Mating Status on Queen Overwintering Survival in the Social Wasp *Vespula maculifrons*

Author(s): Jennifer L. Kovacs and Michael A. D. Goodisman

Source: Environmental Entomology, 41(6):1612-1620. 2012.

Published By: Entomological Society of America

URL: <http://www.bioone.org/doi/full/10.1603/EN12023>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

Effects of Size, Shape, Genotype, and Mating Status on Queen Overwintering Survival in the Social Wasp *Vespula maculifrons*

JENNIFER L. KOVACS AND MICHAEL A. D. GOODISMAN¹

School of Biology, Georgia Institute of Technology, Atlanta, GA 30332

Environ. Entomol. 41(6): 1612–1620 (2012); DOI: <http://dx.doi.org/10.1603/EN12023>

ABSTRACT Many insects survive cold winter temperatures by entering into a transient, dormant state. Social wasp queens in the genus *Vespula* undergo such a state of physiological dormancy known as diapause to survive winter conditions. We experimentally investigated the effects of size, shape, genotype, and matedness on the overwintering survival of *Vespula maculifrons* Buysson (Hymenoptera: Vespidae) queens in two study years. Queens first were mated in the laboratory and then subjected to overwintering conditions. The sizes of several morphological traits of each queen were measured, and the genotype of each queen was determined at polymorphic microsatellite loci allowing queens to be assigned to distinct patriline. We found that overall large body size resulted in high overwintering survival for queens in one study year. In addition, queen thinness was positively and significantly associated with overwintering survival in both years. In contrast, we found no evidence for an effect of patriline on overwintering survival, which is consistent with the idea that natural selection removes genetic variation associated with traits directly linked to fitness. We also found no effect of queen matedness on overwintering survival, as expected under models suggesting that mating should not adversely affect female viability in social hymenopteran insects. Overall, our study demonstrates that some aspects of body size and shape are significantly associated with overwintering survival in *V. maculifrons* queens. However, variation in survival arising from genotypic differences or mating effects is limited due, in part, to the way selection operates in social insect species.

KEY WORDS body size, diapause, hibernation, natural selection, polyandry

Abiotic stress represents an important feature of the environment and may have severe effects on organismal fitness (Hoffmann and Hercus 2000, Badyaev 2005, Kassahn et al. 2009). Exposure to extreme cold, for instance, can substantially limit the ability of an organism to successfully survive and reproduce. Consequently, organisms must respond behaviorally, morphologically, or physiologically if they are to flourish in environments that are persistently cold.

Insects have evolved several physiological and behavioral mechanisms for dealing with cold temperatures (Duman 2001, Bale 2002, Kostal 2006). For example, many insects survive cold weather by entering a dormant state, defined as an adaptive state of developmental arrest. Dormant states include quiescence, whereby a halt in activity is activated by a sudden change in temperature, and diapause, which represents a programmed dormancy typically associated with seasonal changes. The ability to undergo dormancy allows insects to exist in regions with prolonged winters and has been a major factor in the

ecological success of many insect species (Denlinger 2002, Sinclair et al. 2003).

The purpose of this study was to investigate factors affecting the ability of *Vespula* social wasps to survive unfavorably cold temperatures. *Vespula* wasps inhabit regions that experience remarkably cold temperatures. For example, several species can be found near the Arctic Circle (Akre et al. 1980), where winter temperatures fall well below freezing for substantial parts of the year. *Vespula* queens survive cold temperatures throughout their range by overwintering in a state of diapause (Spradbery 1973, Edwards 1980, Greene 1991). They remain in this state while conditions are unfavorably cold and emerge when conditions improve the next spring. Thus, overwintering is a critical aspect of the *Vespula* life cycle.

The focal species of this study, *Vespula maculifrons* (Buysson), commonly known as the eastern yellow-jacket, is found throughout the eastern half of the United States (Akre et al. 1980). *Vespula maculifrons* colonies exhibit an annual life cycle typical of other wasps in the genus. A new colony is founded by a single, multiply-mated queen in the spring (natural history reviewed by Spradbery 1973, Edwards 1980, MacDonald and Matthews 1981, Greene 1991). The queen produces workers throughout the spring and

¹ Corresponding author: M.A.D. Goodisman, School of Biology, Georgia Institute of Technology, 310 Ferst Drive, Atlanta, GA 30332 (e-mail: michael.goodisman@biology.gatech.edu).

summer. At the end of the summer the colony begins to produce males and new reproductive females (unmated queens, known as gynes). In the autumn, gynes participate in mating flights, where they mate with multiple males. The newly mated queens then overwinter for several months. Many queens do not survive this overwintering period. However, the surviving queens emerge in the spring and restart the colony cycle (Ross 1983).

We experimentally determined the effects of several factors on the overwintering survival of *V. maculifrons* queens. First, we investigated if queen overwintering success was linked to body size or shape. Size and shape are important aspects of an individual's phenotype and can have major effects on mating success, fecundity, dispersal, survival, and longevity (Blanckenhorn 2000). For example, female body size is frequently related to female reproductive potential in insects, because larger females are often more fecund or able to produce higher quality offspring than smaller females (Nylín and Gotthard 1998, Blanckenhorn 2005). Queen size also is correlated with colony success in social insects, particularly in species where queens found colonies independently (Keller 1993, Heinze and Tsuji 1995).

We also investigated if queens of different genotypes differed in their ability to survive cold temperatures. *Vespula maculifrons* queens are polyandrous (i.e., they mate with multiple males). Thus, as a group, a queen's female offspring possess substantial genetic diversity (Ross 1983, Goodisman et al. 2007b). Producing a genetically diverse cohort of offspring may provide benefits to the colony under certain conditions (Crozier and Fjerdingstad 2001). Genetic differences among half-sisters within a colony may lead to differences in overwintering survival. However, the presence of genetic variation for a trait like overwintering success is not necessarily expected. Instead, natural selection is predicted to remove genetic variation associated with variation in overwintering survival, because overwintering survival is directly and strongly linked to fitness (Falconer and Mackay 1996).

Finally, we examined the effects that mating had on queen overwintering survival. Mating would seem to be beneficial to both males and females. But the process of mating can have surprisingly negative effects. In particular, males and females can have different genetic interests in polyandrous species and, therefore, come into sexual conflict (Chapman et al. 2003, Arnqvist and Rowe 2005). Such conflict can select for males to harm their mates if such harm allows a male to outcompete his potential rivals (Johnstone and Keller 2000). However, it has been suggested that such conflict should be rare in social hymenopteran insects, such as *V. maculifrons*, where mating happens only at one point in the life cycle and male sperm is used for an extended period of time (Boomsma et al. 2005). Here we tested whether mating affected queen overwintering success in *V. maculifrons*. Overall, our goal was to investigate the effects of size, shape, genotype, and matedness on overwintering survival of *V. maculifrons* and produce a more comprehensive view of the

Table 1. Total number of mated (N_m) and unmated (N_u) gynes from 10 *V. maculifrons* colonies used in mating trials and overwintering experiments

Colony	Latitude, longitude	Date collected	N_m	N_u^a
1	34.588, -84.004	Oct. 18, 2006	0	14 (1)
2	34.588, -84.004	Oct. 18, 2006	1	18 (4)
3	34.588, -84.004	Oct. 18, 2006	2	8 (4)
4	33.871, -84.419	Oct. 19, 2006	0	14 (0)
5	33.871, -84.419	Oct. 19, 2006	3	32 (28)
6	33.926, -84.684	Oct. 19, 2006	2	9 (6)
7	33.871, -84.419	Oct. 19, 2006	1	24 (10)
8	33.766, -84.283	Nov. 3, 2006	6	26 (2)
9	33.737, -84.367	Oct. 25, 2007	26	68
10	33.838, -83.977	Oct. 29, 2007	6	58

^a In 2006, 55 gynes (number per colony given parenthetically) that had not been used in mating trials were used in the overwintering experiments.

factors affecting the success of social insects during this critical dormant period.

Materials and Methods

Mature *V. maculifrons* colonies containing gynes and males were collected from metropolitan Atlanta, GA, during the autumn of 2006 and 2007. We collected gynes from eight colonies in 2006 ($\bar{x} \pm SD$; 20.0 ± 9.6 gynes per colony) and two colonies in 2007 (79.0 ± 21.2 gynes per colony; Table 1). Nonteneral males and gynes were separated from each other and the rest of the colony in the laboratory. All individuals collected were virgins, as mating in *V. maculifrons* takes place outside of the nest under natural conditions (MacDonald and Matthews 1981, Hoffman et al. 2008).

To determine whether mating status (i.e., mated or unmated) affected overwintering survival, we used field-caught gynes and males to conduct mating trials under laboratory conditions (see details provided by Kovacs et al. 2008). All mating trials took place in mating arenas constructed from 10-cm by 10-cm by 8.5-cm Tupperware (Tupperware Brands Corporation, Orlando, FL) containers with a screened-in hole in the lid to allow for airflow. Trials took place indoors under ambient light and at $\approx 20^\circ\text{C}$ air temperature. Males and gynes were marked and numbered so that they could be easily identified. A mean of 1.0 ± 0.2 gynes and 3.1 ± 1.0 nonnestmate males were added to each mating arena in 2006, and a mean of 2.9 ± 1.1 gynes and 5.9 ± 3.8 nonnestmate males were added to each mating arena in 2007. We conducted 829 mating trials in total. In 2006, 168 gynes participated in 377 mating trials, and in 2007, 198 gynes participated in 452 mating trials. Gynes took part in a mean of 2.3 ± 1.6 mating trials in 2006 and 6.2 ± 3.9 mating trials in 2007, which took place over 5.5 wk in 2006 and 2.6 wk in 2007. Mating trials were continuously observed for 45 min or until a male successfully mated with a gyne. Gynes with which a male had successfully inserted for 45 s or longer were considered mated (Kovacs et al. 2008).

Overwintering trials were performed using methods of Ross et al. (1981). We first obtained the wet

mass of the mated and unmated gynes that would be overwintered. All gynes were positioned in ventilated containers filled with moistened paper towels and simultaneously placed at 4°C (Ross et al. 1981, Baer and Schmid-Hempel 2005, Greeff and Schmid-Hempel 2008). This represents a temperature close to the 8.6°C mean temperature experienced for the months of November through April during which *V. maculifrons* gynes overwinter (MacDonald and Matthews 1981). Under these conditions, gynes remained inactive and exhibited a curled posture matching that displayed by naturally overwintering gynes (Spradbery 1973, Edwards 1980). The overwintering gynes were inspected once per week. Any gynes that had died were removed for further analyses. All gynes remained in overwintering conditions until they perished. A proportional hazards model was used to compare survivorship of gynes in 2006 and 2007 (Lawless 1982).

Gynes removed from overwintering trials were photographed immediately in a dorsal view in both years. Individuals were positioned using modeling clay and pins. A Zeiss millimeter micrometer (Carl Zeiss Microimaging, Thornwood, NY) was included in each photo to allow for size calibration. Images were analyzed using tools within the Canvas 9.0.4 computer package (ACD Systems, Victoria, BC, Canada) to obtain measurements of thorax width, thorax length, third tergum length, and gaster length as described by Kovacs et al. (2010a).

Vespa maculifrons queens generally mate with 3–9 males (Ross 1983; Goodisman et al. 2007a,b; Johnson et al. 2009). Consequently, gynes sampled from single colonies belong to multiple patrines. We assigned gynes to patrines within colonies in both study years subsequent to mating and overwintering trials. DNA from gynes was extracted from leg samples by using modifications of the Chelex extraction (Walsh et al. 1991). DNA then was amplified at five highly variable microsatellite markers (Goodisman et al. 2007b) and run on an ABI 3100 sequencer (Life Technologies, Carlsbad, CA). Alleles were visualized using the ABI program Genemapper. Gynes then were assigned to patrines based on their multilocus genotype (Hoffman et al. 2008).

Simple linear regression, as implemented by the computer program JMP version 9.0 (SAS Institute 2010), was used to investigate whether individual size variables could explain the duration of gyne survivorship during overwintering trials. More rigorous multivariate statistical analyses then were used to more accurately dissect the joint influence of morphology, genotype, and matedness on overwintering survival. Before all multivariate analyses, all size measurements were standardized to mean of zero and unit variance within each year. We then used principal components analysis (PCA) to investigate the effects that morphological traits had on overwintering survival (Lande and Arnold 1983, Brodie et al. 1995). PCA was performed on the five standardized morphological traits (thorax width, thorax length, third tergum length, gaster length, and mass) and conducted separately for

each year because of significant differences in eigen-vector loadings between years.

Generalized linear mixed models (GLMMs) were used to determine the effects of gyne size, genotype, and matedness on overwintering survival. GLMMs are appropriate for modeling both fixed and random effects (Bolker 1995, Krackow and Tkadlec 2001, O'Hara 2009), as is the case in our study. In particular, our models incorporated PCs 1–5 and matedness as fixed effects, colony as a random effect, and patriline-within-colony as a nested random effect. The importance of the fixed effects in explaining variation in overwintering survival was determined by the significance of the *t* statistics associated with each fixed factor. To investigate the significance of the random effect of patriline within colony, we determined if a full model that included patriline as a random effect provided a significantly better fit than a nested model excluding patriline as a factor (Pinheiro and Bates 2000). GLMM analyses on overwintering survival were conducted in JMP or using the lmer function of the mle4 package in R version 2.10.0 (R Core Team 2010).

Results

We observed 16 and 36 successful copulations in 2006 and 2007, respectively. In 2006, 14 gynes mated singly and one gyne mated twice, whereas in 2007, 28 gynes mated singly and four gynes mated twice. In 2006, 15 mated and 144 unmated gynes were overwintered. Fifty-five of the unmated gynes in 2006 had not participated in mating trials, and 64 of the gynes used in mating trials were not overwintered. In 2007, 32 mated and 126 unmated gynes were overwintered, and all gynes that were overwintered had participated in mating trials. The number of mating trials in which a gyne participated was unrelated to overwintering viability (2006, $r^2 = 0.006$, $P = 0.42$; 2007, $r^2 = 0.001$, $P = 0.69$) and, therefore, was not considered further.

Gynes gradually perished under overwintering conditions over a period of 11 wk in 2006 ($\bar{x} \pm \text{SD}$; 4.85 ± 2.74) and 20 wk in 2007 (12.48 ± 3.76) (Fig. 1). Mean and variance in gyne survival were significantly greater in 2007 than in 2006 (analysis of variance (ANOVA) test for mean survival, $F = 456.11$; $df = 1, 315$; $P < 0.0001$. Brown–Forsythe test for variance in survival, $F = 22.01$; $df = 1, 156$; $P < 0.0001$). The results of a proportional hazards model revealed strongly significant differences in survivorship in the two study years ($\chi^2 = 220.79$; $df = 1$; $P < 0.0001$). The risk ratio of 10.17 indicated that the risk of a gyne perishing was ≈ 10 -fold greater in 2006 than 2007.

The sizes of the five morphological traits, thorax width, thorax length, third tergum length, gaster length, and mass, were determined for all gynes in both years ($\bar{x} \pm \text{SD}$. Thorax width: 2006, 3.48 ± 0.13 ; 2007, 3.61 ± 0.10 . Thorax length: 2006, 5.22 ± 0.19 ; 2007, 5.13 ± 0.14 . Third tergum length: 2006, 2.66 ± 0.24 ; 2007, 2.41 ± 0.18 . Gaster length: 2006, 9.53 ± 1.20 ; 2007, 10.26 ± 1.52 . Mass: 2006, 180.89 ± 20.01 ; 2007,

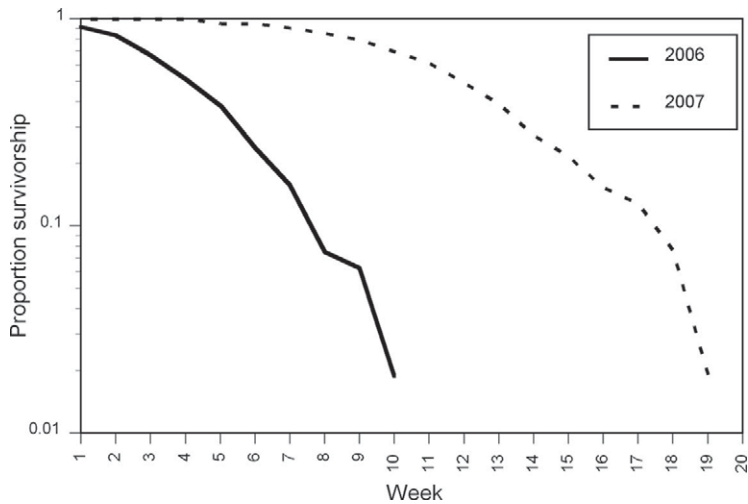


Fig. 1. Survivorship of experimentally overwintered *V. maculifrons* gynes in two study years.

186.69 \pm 18.09). All gyne traits differed significantly in size in the two study years (ANOVA; $P < 0.05$).

We obtained the genotype of a subset of gynes used in the overwintering experiments at several polymorphic microsatellite loci. We assigned paternity to 140 of the 159 gynes in 2006 and 145 of the 158 gynes in 2007. The probability of a male having the same multilocus genotype at these loci was very low ($\ll 0.001$, Goodisman et al. 2007a). Consequently, we were able to readily assign gynes to distinct patrines within colonies. We found that all colonies were headed by a single, multiply-mated queen, as expected (Hoffman et al. 2008; Kovacs et al. 2008, 2010a,b). The mother queens heading the sampled colonies had mated with 4.85 ± 0.90 and 6.00 ± 1.41 males in 2006 and 2007, respectively.

We used univariate linear regressions to gain insight into the morphological factors affecting gyne overwintering survival (Fig. 2). Our univariate analyses revealed that gaster length was significantly and positively correlated with overwintering survival in both study years. In addition, mass and thorax length were significantly and positively correlated with overwintering survival in 2007, but not in 2006. The other measures of gyne size were not significantly correlated with overwintering survival (2006. Thorax width: $F = 0.0041$; $df = 1, 158$; $P = 0.95$; Thorax length: $F = 0.79$; $df = 1, 158$; $P = 0.37$; Third tergum length: $F = 0.12$; $df = 1, 158$; $P = 0.73$; Gaster length: $F = 22.81$; $df = 1, 158$; $P < 0.0001$; Mass: $F = 0.52$; $df = 1, 157$; $P = 0.47$. 2007. Thorax width: $F = 3.27$; $df = 1, 155$; $P = 0.07$; Thorax length: $F = 4.17$; $df = 1, 155$; $P = 0.04$; Third tergum length: $F = 0.14$; $df = 1, 155$; $P = 0.71$; Gaster length: $F = 54.68$; $df = 1, 155$; $P < 0.0001$; Mass: $F = 31.77$; $df = 1, 155$; $P < 0.0001$). Thus, gaster length was a strong predictor of overwintering survival in *V. maculifrons* in both study years, whereas mass and thorax length were predictors of survival in 2007 only.

We then used PCA to more accurately dissect the effects of size, genotype, and matedness on gyne

overwintering survival (Table 2). We found that all size measurements loaded positively on PC1, whereas the size measurements frequently loaded in various positive and negative combinations on the other PCs. Thus, PC1 could be interpreted as quantifying overall body size and the other PCs could be interpreted as measuring different aspects of body shape (Jolicoeur and Mosimann 1960, Cadima and Jolliffe 1996).

We found that several of the PCs derived from gyne morphological trait sizes (Table 2) were associated with overwintering survival (Table 3). In both 2006 and 2007, PC2 and PC5 were significantly associated with gyne survival. PC5 represented a positive effect of gaster length and a negative effect of mass on survival. Similarly, PC2 represented a positive effect of gaster length and a negative effect of thorax size on overwintering survival. Therefore, in both study years, the significance of PC2 and PC5 reflected a positive association between survival and gyne "thinness." This interpretation was supported by strong positive associations between direct measures of thinness, such as the ratio of gaster length to mass (GL/M) or gaster length to thorax width (GL/TW), and survivorship (S) in both years (2006. GL/M versus S, $\rho = 0.448$; GL/TW versus S, $\rho = 0.383$. 2007. GL/M versus S, $\rho = 0.370$; GL/TW versus S, $\rho = 0.486$. $P < 0.0001$ for all Spearman's correlations).

In 2007, but not in 2006, PC1 and PC3 were significantly associated with overwintering survival (Table 3). PC3 primarily represented an effect of third tergum length on overwintering survival. In contrast, PC1 indicated a positive effect of gyne size on overwintering survival. Thus, overall, our multivariate analyses indicated that thin gynes survived cold temperatures particularly well in both years. In addition, large gynes survived cold temperatures better than small gynes in 2007.

To determine if genetic differences among gynes within colonies (i.e., gynes from different patrines)

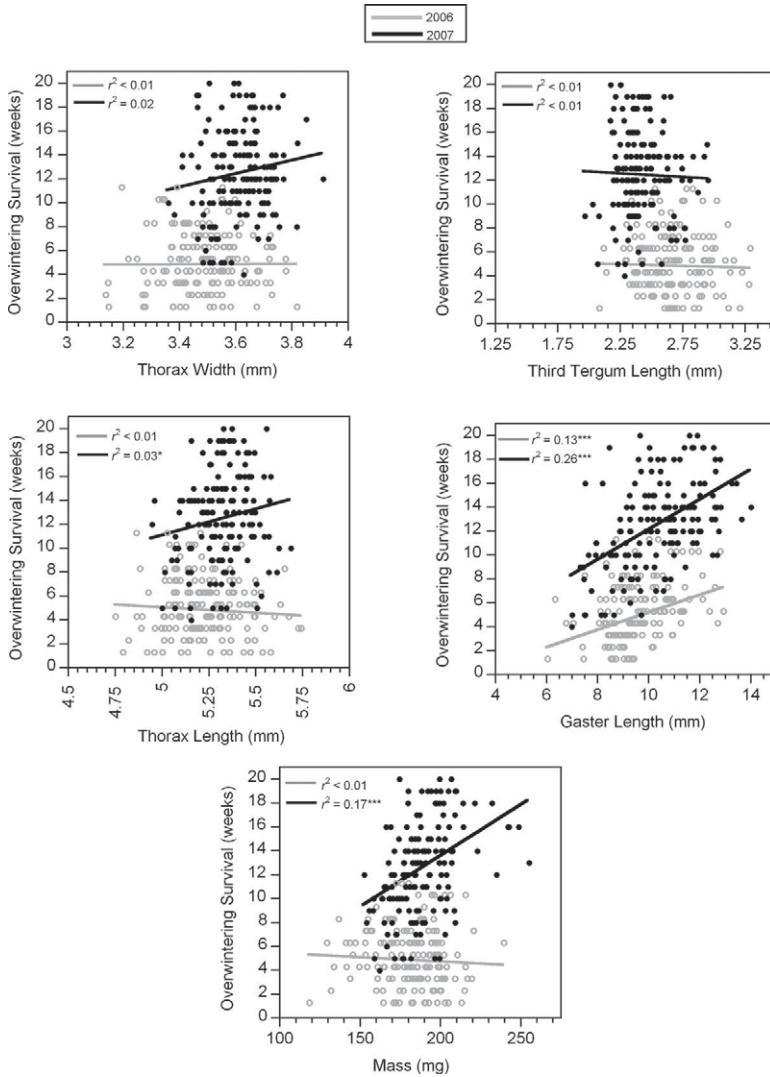


Fig. 2. Relationship between overwintering survival and trait size of *V. maculifrons* gynes. r^2 values significant at * $P < 0.05$ or *** $P < 0.001$.

were associated with differences in overwintering survival, we compared the fit of a full GLMM model that included the five PCs, matedness, and patriline against a model that included only the five PCs and matedness. We found no significant difference in fit between these models in either year ($P > 0.7$, Table 4). This indicated that there were no significant differences in the viability of gynes from different patrilines in our study system.

Finally, we determined if mated and unmated gynes differed in overwintering success. We found that gyne matedness was not a significant factor in predicting overwintering survival in either 2006 or 2007 (Table 3). Thus, overall, our data suggested that some aspects of gyne size and shape significantly influenced overwintering survival. However, genotype and mating status did not significantly affect gyne viability.

Discussion

Gyne Size and Shape Influence Overwintering Survival. We detected significant effects of *V. maculifrons* gyne morphology on overwintering survival in both study years. Specifically, gyne thinness was positively correlated with overwintering survival in both 2006 and 2007 (Table 2). In contrast, in 2007, but not 2006, there was a strong positive effect of overall body size on survival (Fig. 2; Table 3).

The differences between the 2006 and 2007 data could have several nonmutually exclusive origins. First, colony effects may have influenced overwintering effects. Indeed, gyne survival was significantly lower in 2006 than 2007 (Fig. 1). The origin of these differences remains unclear. However, the colonies sampled in the two study years were genetically dis-

Table 2. Principal components (PCs) describing the relationships among five morphological traits in *V. maculifrons* gynes

	Year	PC1	PC2	PC3	PC4	PC5
		Total percent variance explained				
	2006	44	23	16	10	7
	2007	37	24	19	15	5
		Eigenvalues				
Traits						
Thorax width	2006	0.46	0.46	0.01	-0.76 ^a	0.09
	2007	0.32	0.63 ^a	-0.22	0.67 ^a	0.04
Thorax length	2006	0.34	0.68 ^a	-0.08	0.63 ^a	0.09
	2007	0.41	0.51 ^a	-0.20	-0.73 ^a	-0.11
Third tergum length	2006	0.37	-0.20	0.89 ^a	0.13	0.09
	2007	0.30	0.16	0.94 ^a	0.01	0.05
Gaster length	2006	0.48	-0.45	-0.38	0.10	0.64 ^a
	2007	0.60 ^a	-0.45	-0.08	0.14	-0.64 ^a
Mass	2006	0.55 ^a	-0.28	-0.23	0.08	-0.75 ^a
	2007	0.53 ^a	-0.35	-0.15	-0.02	0.75 ^a

Each eigenvalue represents the eigenvectors of trait sizes in the five PCs, which were used in the multivariate models.

^a Eigenvalues with magnitudes of >0.5 or less than -0.5 in each year.

tinct and had experienced different environmental conditions before collection (Table 1). Thus, differences in the factors affecting overwintering of queens between years may have been associated with variation in the timing or location of collection, differences in weather conditions between years, or differential physiological preparedness for overwintering of gynes resulting from genetic or environmental factors.

Second, the between-year variation in how body size measurements influenced overwintering survival may have reflected how different size measures were associated with survivorship over time. Gyne thinness was significantly associated with overwintering survival over the first 11 wk of the 2007 trials ($P < 0.01$), as was the case with the 2006 data. In contrast, overall body size was not associated with survival ($P > 0.1$) in the first 11 wk of 2007 and only became significant when the entire 20-wk period was considered. Thus it appears that overall size effects on survival are not manifested until gynes have been in diapause for some time, and gynes may not have survived long enough in 2006 for these effects to be observed.

It is also important to note that our experimental treatment may not have mimicked exactly how gynes enter their overwintering phase. True hibernation in *Vespa* may require a programmed process involving several stages that allows the insect to prepare for the overwintering period (Kostal 2006). Our experimental gynes were sampled during the period when they would have been preparing for hibernation. However, we cannot guarantee that the subjects were com-

pletely prepared for the overwintering process when the experiments commenced, even though overwintering gynes in this study displayed the characteristic pose of naturally hibernating gynes in the field (Spradbery 1973). Consequently, the fact that laboratory overwintering experiments necessarily differed from how gynes undergo hibernation in nature may have affected our results to some degree. Regardless, our finding of differences in how some size measurements affected overwintering survival in the two study years demonstrates the importance of conducting multiple seasons of analyses; studies undertaken in only a single season may arrive at incorrect conclusions regarding what factors affect survival.

Our overall assessment is that some aspects of size and shape influence gyne overwintering success in *V. maculifrons*. Our finding that larger gynes enjoyed greater overwintering success than smaller gynes in 2007 is in line with many previous studies in insects that examined the effect of female size on survival (Stearns 1992, Reeve and Fairbairn 1999, Burkhard et al. 2002, Hahn et al. 2008). Although small female body size is associated with increased fecundity in some insects, this is generally because of the allocation of resources toward egg-production, which often decreases longevity (Banks and Thompson 1987, Jervis et al. 2001, Thorne et al. 2006). Thus, large size is likely to be an important factor in determining high viability in many insects.

Moreover, queen size has been found to be an important factor affecting queen viability in social insects. For example, queen size is positively associated with queen survival during mating flights or col-

Table 3. Effects of five principal components (PCs) derived from *V. maculifrons* gyne size measurements (Table 2) and gyne matedness on overwintering survival

Variable	2006	2007
PC1	$t_{125} = 1.77$	$t_{137} = 6.67^{***}$
PC2	$t_{118} = -2.24^*$	$t_{137} = -2.38^*$
PC3	$t_{128} = -1.61$	$t_{137} = -2.80^{**}$
PC4	$t_{129} = 0.58$	$t_{133} = 0.38$
PC5	$t_{132} = 5.59^{***}$	$t_{150} = -2.88^{**}$
Matedness	$t_{130} = -0.05$	$t_{123} = 0.07$

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 4. Comparisons of full models, incorporating the effects of gyne size, mating status, and patriline, and nested models, incorporating the effects of gyne size and mating status only

Year	Model	Log-likelihood	c ² for model comparison	P
2006	Full	-337.52	0.247	0.884
	Nested	-337.65		
2007	Full	-400.97	0.615	0.735
	Nested	-401.28		

only founding in some ants (Wiernasz and Cole 2003, Fjerdingstad 2005). In addition, heavier *Polistes chinensis* (Fabricius) paper wasp foundresses construct larger nests than smaller foundresses (Kudo et al. 2005). Queen size also affects dispersal abilities or behaviors in many social insects; large queens generally disperse further and tend to found colonies independently more often than small queens (Keller and Passera 1989, Sundström 1995, Rüppell et al. 1998, Rüppell and Heinze 1999, Peeters and Ito 2001). It thus seems reasonable to conclude that queen size frequently directly impacts queen survival. Such an advantage may naturally arise from having increased body reserves or mass, which may permit queens to better survive variable environmental conditions.

Despite the seeming importance of overall gyne body size, our most consistent result was that gyne thinness was associated positively with overwintering survival in both 2006 and 2007. This surprising finding suggests that gynes of certain shapes survive overwintering well. The reason why thin gynes were more capable of surviving overwintering is not completely clear, but may reflect certain aspects of gyne metabolism. In addition, previous work suggests that thorax size is under stronger selection in *V. maculifrons* gynes than in workers, and that gyne thoraces are significantly smaller than expected in proportion to their overall body size (negatively allometric; Kovacs et al. 2010a). In light of our current findings, it would appear that overwintering plays a role in generating some of the selective pressures responsible for the significant morphological differences observed between *V. maculifrons* workers and gynes (Kovacs et al. 2010a).

Genotype Does Not Influence Gyne Overwintering Survival. We failed to detect an effect of genotype on overwintering survival in either 2006 or 2007. That is, gynes sired by different males did not differ in their overwintering success. Consequently, the genetic diversity among offspring generated by polyandrous mating behavior in *V. maculifrons* did not lead to variation in overwintering survival among half-sibs in our study.

Genetic variation associated with fitness-related traits can be maintained under some circumstances (Merila and Sheldon 1999). However, natural selection should act to remove genetic variation for traits that are strongly linked to fitness under simple conditions (Mousseau and Roff 1987). Overwintering survival is clearly and directly linked to queen fitness, and is therefore expected to be under strong directional selection. Thus, the lack of substantial genetic variation associated with variation in survival is not unexpected. Alternatively, genetic effects on overwintering survival may be present but limited in magnitude in *V. maculifrons*, and therefore require larger sample sizes to be readily detected.

Mating Status Does Not Influence Gyne Overwintering Survival. Mating can have a negative impact on female survival if males transfer seminal toxins during mating or otherwise injure females during copulation (Eberhard 1996, Johnstone and Keller 2000, Simmons 2001). Injuring of females through mating can be

adaptive if males increase their reproductive success by outcompeting other males. However, we discovered no significant association between gyne matedness and overwintering survival in either study year. This result is consistent with findings of Ross et al. (1981), who found that unmated *V. germanica* queens successfully overwintered and subsequently attempted to initiate nests.

The lack of an association between overwintering survival and matedness may arise because males are selected to not harm their mates when there is a long delay between mating and the production of offspring (Strassmann 2001). Such is the case in *V. maculifrons*, where queens must survive months before they produce their first cohort of new sexuals. Though short-term strategies to prevent female remating such as mating plugs have evolved in some social insect species (Baer et al. 2001, Mikheyev 2003, Boomsma et al. 2005), strategies to prevent female promiscuity with long-term detrimental effects on the female should be constrained, because males may decrease their own fitness by decreasing the longevity of their mate (Boomsma and Ratnieks 1996, Strassmann 2001). We also cannot rule out the possibility that we failed to detect a strong signal of the effects of queen matedness because our sample sizes of mated queens were somewhat modest.

Empirical studies investigating the effects of mating on queen survival have returned mixed results. In the bumblebee *Bombus terrestris* (Linnaeus), virgin gynes survived overwintering longer than mated gynes (Greeff and Schmid-Hempel 2008). In addition, *B. terrestris* gynes artificially inseminated with sperm from multiple males suffered from higher mortality during overwintering than those artificially inseminated with sperm from a single male, suggesting a negative effect of multiple mating on queen viability (Baer 2005). In contrast, *Cardiocondyla obscurior* (Wheeler) ant queens that mated with either one fertile or one sterilized male had greater longevity than unmated queens, indicating that mating is actually beneficial to queens in this species (Schrepf et al. 2005). Thus the effects of mating on queen survival seem to have different effects in different social insect taxa.

Conclusions. Behavioral and physiological responses to cold environmental temperatures are fundamental to the success of many insects. In this study, we investigated factors associated with the survival of *V. maculifrons* wasp queens in persistent cold temperatures. We discovered that body size and shape, which are among the most important aspects of individual phenotype, displayed important associations with survival. Thus, natural selection likely directly operates on queen size and shape in this species through its effects on overwintering success. In contrast, we failed to detect an effect of mating and genotype on overwintering survival. These latter findings are consistent with the effects of selection pressures operating on social insect queens and further demonstrate the idiosyncratic way that selection may operate in social insects.

Acknowledgments

We thank E. L. Johnson, T. W. Cunningham, S. M. Marriner, D. B. Bhatka, and E. A. Matthews for their generous lab support; and B. G. Hunt and four anonymous reviewers for helpful comments, statistical advice, and discussion during the preparation of this manuscript. This work was supported by the Georgia Institute of Technology and the United States National Science Foundation (grants DEB-0640690 and IOS-0821130).

References Cited

- Akre, R. D., A. Greene, J. F. MacDonald, P. J. Landolt, and H. G. Davis. 1980. The yellow jackets of America north of Mexico. Agriculture Handbook 552, U. S. Department of Agriculture.
- Arnqvist, G., and L. Rowe. 2005. Sexual conflict. Princeton University Press, Princeton, NJ.
- Badyaev, A. V. 2005. Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. *Proc. R. Soc. Lond. B* 272: 877–886.
- Baer, B. 2005. Sexual selection in *Apis* bees. *Apidologie* 36: 187–200.
- Baer, B., and P. Schmid-Hempel. 2005. Sperm influences female hibernation success, survival and fitness in the bumble-bee *Bombus terrestris*. *Proc. R. Soc. Lond. B* 1560: 319–323.
- Baer, B., E. D. Morgan, and P. Schmid-Hempel. 2001. A nonspecific fatty acid within the bumblebee mating plug prevents females from remating. *Proc. Natl. Acad. Sci. U.S.A.* 98: 3926–3928.
- Bale, J. S. 2002. Insects and low temperatures: from molecular biology to distributions and abundance. *Philos. Trans. R. Soc. Lond. B* 357: 849–861.
- Banks, M. J., and D. J. Thompson. 1987. Lifetime reproductive success of females of the damselfly *Coenagrion puella*. *J. Anim. Ecol.* 56: 815–832.
- Blanckenhorn, W. U. 2000. The evolution of body size: what keeps organisms small? *Q. Rev. Biol.* 75: 385–407.
- Blanckenhorn, W. U. 2005. Behavioral causes and consequences of sexual size dimorphism. *Ethology* 111: 977–1016.
- Bolker, J. A. 1995. Model systems in developmental biology. *BioEssays* 17: 451–455.
- Boomsma, J. J., and F.L.W. Ratnieks. 1996. Paternity in eusocial Hymenoptera. *Philos. Trans. R. Soc. Lond. B* 351: 947–975.
- Boomsma, J. J., B. Baer, and J. Heinze. 2005. The evolution of male traits in social insects. *Annu. Rev. Entomol.* 50: 395–420.
- Brodie, E. D., A. J. Moore, and F. J. Janzen. 1995. Visualizing and quantifying natural selection. *Trends Ecol. Evol.* 10: 313–318.
- Burkhard, D. U., P. I. Ward, and W. U. Blanckenhorn. 2002. Using age grading by wing injuries to estimate size-dependent adult survivorship in the field: a case study of the yellow dung fly *Scathophaga stercoraria*. *Ecol. Entomol.* 27: 514–520.
- Cadima, J.F.C.L., and I. T. Jolliffe. 1996. Size- and shape-related principal component analysis. *Biometrics* 52: 710–716.
- Chapman, T., G. Arnqvist, J. Bangham, and L. Rowe. 2003. Sexual conflict. *Trends Ecol. Evol.* 18: 41–47.
- Crozier, R. H., and E. J. Fjerdingstad. 2001. Polyandry in social Hymenoptera-disunity in diversity? *Annals Zoologici Fennici* 38: 267–285.
- Denlinger, D. L. 2002. Regulation of diapause. *Annu. Rev. Entomol.* 47: 93–122.
- Duman, J. G. 2001. Antifreeze and ice nucleator proteins in terrestrial arthropods. *Annu. Rev. Physiol.* 63: 327–357.
- Eberhard, W. G. 1996. Female control: sexual selection by cryptic female choice. Princeton University Press, Princeton, NJ.
- Edwards, R. E. 1980. Social wasps: their biology and control. Rentokil, East Grinstead, United Kingdom.
- Falconer, D. S., and T.F.C. Mackay. 1996. Quantitative genetics. Pearson Education Ltd, Essex, United Kingdom.
- Fjerdingstad, E. J. 2005. Control of body size of *Lasius niger* ant sexuals-worker interests, genes and environment. *Mol. Ecol.* 14: 3123–3132.
- Goodisman, M.A.D., J. L. Kovacs, and E. A. Hoffman. 2007a. Lack of conflict during queen production in the social wasp *Vespula maculifrons*. *Mol. Ecol.* 16: 2589–2595.
- Goodisman, M.A.D., J. L. Kovacs, and E. A. Hoffman. 2007b. The significance of multiple mating in the social wasp *Vespula maculifrons*. *Evolution* 61: 2260–2267.
- Greeff, M., and P. Schmid-Hempel. 2008. Sperm reduces female longevity and increases melanization of the spermatheca in the bumblebee *Bombus terrestris* L. *Insectes Soc.* 55: 313–319.
- Greene, A. 1991. *Dolichovespula* and *Vespula*, pp. 263–305. In K. G. Ross and R. W. Matthews (eds.), The social biology of wasps. Comstock Publishing Associates, Ithaca, NY.
- Hahn, D. A., A. R. Martin, and S. D. Porter. 2008. Body size, but not cooling rate, affects supercooling points in the red imported fire ant, *Solenopsis invicta*. *Environ. Entomol.* 37: 1074–1080.
- Heinze, J., and K. Tsuji. 1995. Ant reproductive strategies. *Res. Popul. Ecol.* 37: 135–149.
- Hoffmann, A. A., and M. J. Hercus. 2000. Environmental stress as an evolutionary force. *Bioscience* 50: 217–226.
- Hoffman, E. A., J. L. Kovacs, and M.A.D. Goodisman. 2008. Genetic structure and breeding system in a social wasp and its social parasite. *BMC Evol. Biol.* 8: Article 239.
- Jervis, M. A., G. E. Heimpel, P. N. Ferns, J. A. Harvey, and N.A.C. Kidd. 2001. Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. *J. Anim. Ecol.* 70: 442–458.
- Johnson, E. L., T. W. Cunningham, S. M. Marriner, J. L. Kovacs, B. G. Hunt, D. B. Bhatka, and M.A.D. Goodisman. 2009. Resource allocation in a social wasp: effects of breeding system and life cycle on reproductive decisions. *Mol. Ecol.* 18: 2908–2920.
- Johnstone, R. A., and L. Keller. 2000. How males can gain by harming their mates: sexual conflict, seminal toxins, and the cost of mating. *Am. Nat.* 156: 368–377.
- Jolicoeur, P., and J. E. Mosimann. 1960. Size and shape variation in the painted turtle. A principal component analysis. *Growth* 24: 339–354.
- Kassahn, K. S., R. H. Crozier, H. O. Portner, and M. J. Caley. 2009. Animal performance and stress: responses and tolerance limits at different levels of biological organisation. *Biol. Rev.* 84: 277–292.
- Keller, L. 1993. Queen number and sociality in insects. Oxford University Press, Oxford, United Kingdom.
- Keller, L., and L. Passera. 1989. Size and fat content of gynes in relation to the mode of colony founding in ants (Hymenoptera: Formicidae). *Oecologia* 80: 236–240.
- Kostal, V. 2006. Eco-physiological phases of insect diapause. *J. Insect Physiol.* 52: 113–127.
- Kovacs, J. L., E. A. Hoffman, and M.A.D. Goodisman. 2008. Mating success in the polyandrous social wasp *Vespula maculifrons*. *Ethology* 114: 340–350.

- Kovacs, J. L., E. A. Hoffman, S. M. Marriner, and M.A.D. Goodisman. 2010a. Detecting selection on morphological traits in social insect castes: the case of the social wasp *Vespula maculifrons*. *Biol. J. Linn. Soc.* 101: 93–102.
- Kovacs, J. L., E. A. Hoffman, S. M. Marriner, J. A. Rekau, and M.A.D. Goodisman. 2010b. Environmental and genetic influences on queen and worker body size in the social wasp *Vespula maculifrons*. *Insectes Soc.* 57: 53–65.
- Krackow, S., and E. Tkadlec. 2001. Analysis of brood sex ratios: implications of offspring clustering. *Behav. Ecol. Sociobiol.* 50: 293–301.
- Kudo, K., S. Tsujita, K. Tsuchida, W. Goi, S. Yamane, S. Mateus, Y. Ito, S. Miyano, and R. Zucchi. 2005. Stable relatedness structure of the large-colony swarm-founding wasp *Polybia paulista*. *Behav. Ecol. Sociobiol.* 58: 27–35.
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37: 1210–1226.
- Lawless, J. F. 1982. The statistical models and methods for lifetime data. Wiley, New York.
- MacDonald, J. F., and R. W. Matthews. 1981. Nesting biology of the eastern yellowjacket, *Vespula maculifrons* (Hymenoptera: Vespidae). *J. Kans. Entomol. Soc.* 54: 433–457.
- Merila, J., and B. C. Sheldon. 1999. Genetic architecture of fitness and nonfitness traits: empirical patterns and development of ideas. *Heredity* 83: 103–109.
- Mikheyev, A. S. 2003. Evidence for mating plugs in the fire ant *Solenopsis invicta*. *Insectes Soc.* 50: 401–402.
- Mousseau, T. A., and D. A. Roff. 1987. Natural selection and the heritability of fitness components. *Heredity* 59: 181–197.
- Nylin, S., and K. Gotthard. 1998. Plasticity in life-history traits. *Annu. Rev. Entomol.* 43: 63–83.
- O'Hara, R. B. 2009. How to make models add up - a primer on GLMMs. *Ann. Zool. Fenn.* 46: 124–137.
- Peeters, C., and F. Ito. 2001. Colony dispersal and the evolution of queen morphology in social Hymenoptera. *Annu. Rev. Entomol.* 46: 601–630.
- Pinheiro, J. C., and D. M. Bates. 2000. Mixed-effects models in S and S-PLUS. Springer, New York.
- R Core Team. 2010. R: a language and environment for statistical computing. Vienna, Austria.
- Reeve, J. P., and D. J. Fairbairn. 1999. Change in sexual size dimorphism as a correlated response to selection on fecundity. *Heredity* 83: 697–706.
- Ross, K. G. 1983. Laboratory studies of the mating biology of the eastern yellowjacket, *Vespula maculifrons* (Hymenoptera: Vespidae). *J. Kans. Entomol. Soc.* 56: 523–537.
- Ross, K. G., R. W. Matthews, and R. A. Morse. 1981. Laboratory culture of four species of yellowjackets, *Vespula* spp. Foundress nest initiation. *Ann. Entomol. Soc. Am.* 74: 247–254.
- Rüppell, O., and J. Heinze. 1999. Alternative reproductive tactics in females: the case of size polymorphism in winged ant queens. *Insectes Soc.* 46: 6–17.
- Rüppell, O., J. Heinze, and B. Hölldobler. 1998. Size-dimorphism in the queens of the North American ant *Leptothorax rugatulus* (Emery). *Insectes Soc.* 45: 67–77.
- SAS Institute. 2010. JMP user's guide. SAS Institute, Cary, NC.
- Schrempf, A., J. Heinze, and S. Cremer. 2005. Sexual cooperation: mating increases longevity in ant queens. *Curr. Biol.* 15: 267–270.
- Simmons, L. W. 2001. Sperm competition and its evolutionary consequences in the insects. Princeton University Press, Princeton, NJ.
- Sinclair, B. J., P. Vernon, C. J. Klok, and S. L. Chown. 2003. Insects at low temperatures: an ecological perspective. *Trends Ecol. Evol.* 18: 257–262.
- Spradbery, J. P. 1973. Wasps: an account of the biology and natural history of solitary and social wasps. Sidgwick & Jackson, London, United Kingdom.
- Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, Oxford, United Kingdom.
- Strassmann, J. 2001. The rarity of multiple mating by females in the social Hymenoptera. *Insectes Soc.* 48: 1–13.
- Sundström, L. 1995. Sex allocation and colony maintenance in monogyne and polygyne colonies of *Formica truncorum* (Hymenoptera: Formicidae): the impact of kinship and mating structure. *Am. Nat.* 146: 182–201.
- Thorne, A. D., J. J. Pexton, C. Dytham, and P. J. Mayhew. 2006. Small body size in an insect shifts development, prior to adult eclosion, towards early reproduction. *Proc. R. Soc. B* 273: 1099–1103.
- Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10: 506–513.
- Wiernasz, D. C., and B. J. Cole. 2003. Queen size mediates queen survival and colony fitness in harvester ants. *Evolution* 57: 2179–2183.

Received 23 January 2012; accepted 26 July 2012.