Sex allocation ratios in the facultatively polygynous ant, *Leptothorax acervorum*

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Abstract:
We investigated sex allocation in a central European population of the facultatively polygynous ant *Leptothorax acervorum*. The population-wide sex ratio was found to be quite balanced, with a proportional investment in female sexuals of 0.49. Sex allocation varied considerably between colonies, resulting in split sex ratios. The productivity of colonies was negatively correlated with queen number and positively with colony size. In contrast, the sex ratio (proportional investment in female sexuals) was neither correlated with queen number, colony size, nor total sexual production, but with worker relatedness. The uncoupling of the genetic colony structure and queen number presumably results from frequent queen turnover and colony splitting.
Keywords. Sex ratio - Relatedness - Relative relatedness asymmetry - Polygyny – Formicidae

Article:
Introduction
Populations of many animals consist of equal numbers of males and females. The occurrence of balanced sex ratios in some species with environmental sex determination (e.g., Bull and Charnov *1988*), and skewed sex ratios in birds with sex chromosomes (e.g., Komdeur et al. *1997*) indicates that this 1:1 sex ratio is frequently not a simple consequence of the mechanisms of sex determination. Instead, Fisher (*1930*) argued that a population-wide 1:1 sex investment ratio is an evolutionarily stable strategy, because only then do the individuals that control the investment obtain equal fitness payoffs from both sexes.

Sex allocation in social Hymenoptera (bees, wasps, and ants) is of special interest, because the sex investment of a colony can be controlled by two parties, the queen and her helping daughters (workers), whose fitness interests may differ: the mother queen is equally related to her sons and daughters, but workers in colonies with one singly mated queen (monogyny, monandry) are three times more closely related to female sexuals than to males (relatedness asymmetry). Whereas the queen therefore favors equal investment in male and female sexuals, workers benefit most from investing three times more in female than male sexuals. The finding that, on average, population-wide sex investment ratios in monogynous, monandrous ants are female-biased, strongly

suggests worker control (Trivers and Hare *1976*; Bourke and Franks *1995*; Crozier and Pamilo *1996*; Chapuisat and Keller *1999*).

However, the pattern of sex investment in social Hymenoptera is often more complex. For example, in many species, colonies are not obligatorily monogynous but some nests may have several related queens (facultative polygyny). The workers' relatedness asymmetry at the colony level is expected to decline with increasing queen number per colony and, as a consequence, a negative correlation of colony queen number and female sex investment is predicted under the relative relatedness asymmetry hypothesis (Boomsma and Grafen *1990*, *1991*). Several studies have clearly demonstrated this effect (Boomsma *1993*; Bourke and Franks *1995*; Crozier and Pamilo *1996*).

Leptothorax acervorum is a facultatively polygynous ant, whose colonies consist only of a few dozen workers which nest in rotting branches and under bark. Queens typically mate singly (Heinze et al. *1995*), and in colonies containing a queen, workers rarely lay eggs and, therefore, presumably do not contribute considerably to the colonies' males (Bourke *1991*; Heinze et al. *1997a*). Previous investigations revealed marked differences in sex allocation patterns between populations of this ant: in an English population (Chan and Bourke *1994*; Chan et al. *1999*), an effect of queen number on colony sex ratio was demonstrated, and allozyme data suggested that virgin queens in the Reichswald population in southern Germany are predominantly reared in nests with high relatedness (Heinze et al. *1995*). Here, employing high-resolution genetic markers (microsatellites), we show that sex investment ratios in the Reichswald population vary with average worker relatedness but not with queen number, presumably due to rapid changes in the social structure of colonies.

Methods
Colonies of *L. acervorum* were collected from their nests in rotting pine twigs in an open pine forest, in Nürnberger Reichswald near Erlangen in early summer 1998. Colonies were kept in the laboratory as previously described (Heinze and Ortius *1991*) until no more sexuals eclosed from the brood. New workers eclosed over a prolonged period of time and their production was presumably influenced by rearing conditions in the laboratory. Hence, the total productivity of colonies was not examined.

The dry weights of female and male sexuals of *L. acervorum* do not differ significantly (Chan and Bourke *1994*; Heinze et al. *1995*), so the sex ratio (defined as the proportional investment in virgin queens) was therefore estimated as the ratio of female sexuals to the total number of sexuals. We investigated the effects of parameters which have previously been discussed as influential on sex ratio (queen number, worker number, and total sexual production) and total sexual production (queen number and worker number). The distribution of data was normalized by $\log_{10}(x+0.5)$ transformation (Kolmogorov-Smirnov test; queen number, $D=0.167$, $P>0.20$; worker number, $D=0.054$, $P>0.20$; total sexual production, $D=0.119$, $P>0.20$) and angular transformation (sex ratios, $D=0.142$, $P>0.20$). Relatedness was normally distributed ($D=0.141$, $P>0.20$).

Transformed data were analyzed by parametric multiple regression. Because of the presumed interrelations between the various "independent variables," in addition we analyzed the data by

standard methods (James and McCulloch *1990*), employing bootstrapping for significance tests (Manly *1997*; O. Rüppell, J. Heinze, B. Hölldobler, unpublished data).

DNA for genetic analyses was extracted from adult ants using Chelex 100 following a protocol by Thorén et al. (*1995*). Microsatellites L18 (Foitzik et al. *1997*) and LXGT 223 (Hamaguchi et al. *1993*) were amplified in 25-µl reaction volumes [5.0 µl DNA, 2.5 µl 10xPCR buffer (MBI Fermentas), 16.7 pmol of each primer, 4.5 µl 4 mM dNTPs, 6.55 µl distilled water, 2.25 µl 25 mM $MgCl_2$, and 0.2 µl (5 units/µl) *Taq* polymerase (MBI Fermentas)] using a Perkin Elmer GeneAmp 2400 or MWG Primus 96plus thermocycler. The temperature profile started with 5 min at 94°C, followed by 32 cycles of 1 min 94°C, 1 min 54°C, and 1 min 72°C, and ended with 7 min at 72°C. PCR products were separated on 40-cm-long non-denaturing 10% polyacrylamide gels for 3 h at 40 W, stained with Sybr Green (5 µl in 50 ml 1xTBE buffer), and visualized under UV light. Allele size was determined by comparison with a pUC19 DNA/*Msp*I (*Hpa*II) size marker (MBI Fermentas). Within-colony worker relatedness was calculated from microsatellite genotypes with RELATEDNESS 4.2 (K.F. Goodnight and D.C. Queller, based on Queller and Goodnight *1989*) and used as an indicator for worker relatedness asymmetry because direct determination of the latter was precluded by low overall sexual production.

Results

Of 62 collected colonies, 46 were polygynous (median three queens, range two to ten), 15 were monogynous, and 1 colony was queenless. The number of workers per colony ranged from 12 to 157 (median 42.5). Ten colonies did not produce sexuals. In the remaining 52 colonies, a total of 287 virgin queens and 298 males were produced (proportion of females 0.49, confidence interval 0.39-0.59, estimated following Bourke and Franks *1995*). The colony-level proportional investment in female sexuals was bimodally distributed (Fig. 1). Twelve colonies (23%) produced only female sexuals (median 2.5, range 1-59) and 13 colonies (25%) produced only male sexuals (median 5, range 1-23). A total of 102 female sexuals (35%) and 105 males (35%) were produced in colonies with single-sex broods.
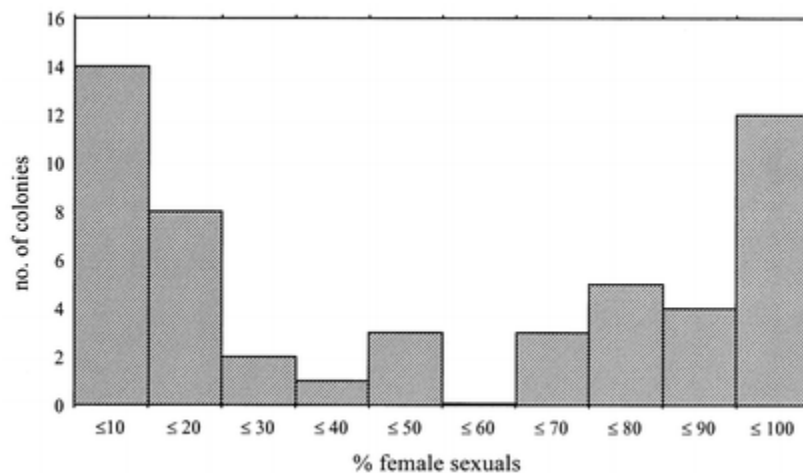

Fig. 1. Between-colony variation in proportional investment in female sexuals in the Reichswald population of the facultatively polygynous ant, *Leptothorax acervorum*. Proportional investment is bimodally distributed, with some colonies producing only male sexuals and others only female sexual

In a multiple-regression analysis, log-transformed total sexual production was found to be positively correlated with log-transformed worker number and negatively with log-transformed queen number (Table 1). Worker number and total sexual production were also correlated in a univariate analysis, whereas the correlation between queen number and total sexual production was not significant (Table 1).

Table 1 Correlation between queen number, worker number, total sexual production and sex ratio in the ant, *Leptothorax acervorum*. Data were analyzed by multiple regression after log or angular transformation (β-values ±SE) and by univariate, parametric correlation (r values) with bootstrapping (95% confidence intervals of r are given in *parentheses*). After Bonferroni correction for multiple testing, the results of univariate correlations are significant at a 5% level only with $P<0.005$ (*n.a.* not analyzed). Relatedness was investigated by genetic markers in only a subset of 20 colonies

Dependent variable	Independent variables				Multiple regression result
	Queen number	Worker number	Total sexual production	Relatedness	
Total sexual production ($n=62$)	β=−0.254±0.101 t_{59}=−2.590, P=0.015 r=−0.152 (−0.359–0.136), P=0.244	β=0.571±0.101 t_{59}=5.633, P=0.0001 r=0.568 (0.367–0.721), $P<0.001$		n.a.	R=0.628 $F_{2,59}$=19.212 $P<0.00001$
Sex ratio ($n=52$)	β=−0.056±0.142 t_{48}=−0.394, P=0.695 r=−0.030 (−0.366–0.224), P=0.834	β=−0.279±0.167 t_{48}=−1.667, P=0.102 r=−0.205 (−0.468–0.077), P=0.142	β=0.043±0.169 t_{48}=0.257, P=0.798 r=0.034 (−0.248–0.279), P=0.819	n.a.	R=0.269 $F_{3,48}$=1.244 $P<0.304$
Sex ratio ($n=20$)	β=−0.058±0.203 t_{15}=−0.287, P=0.778 r=−0.312 (−0.617–0.046), P=0.186	β=−0.431±0.216 t_{15}=−1.994, P=0.065 r=−0.347 (−0.783–0.100), P=0.145	β=0.230±0.222 t_{15}=1.035, P=0.317 r=0.228 (−0.226–0.575), P=0.313	β=0.451±0.208 t_{15}=2.163, P=0.047 r=0.550 (0.150–0.873), P=0.012	R=0.703 $F_{4,15}$=3.659 $P<0.0285$

Sex ratio (proportional investment in female sexuals) neither varied significantly with queen number, worker number, nor total sexual production (Table 1). Monogynous and polygynous colonies did not differ in their mean proportional investment in females (36 polygynous colonies: median 0.333; 15 monogynous colonies: 0.568, Mann-Whitney U-test, U=247.5, P=0.64).

For investigation of within-colony worker relatedness, only colonies that had produced more than ten sexuals were included (5 monogynous, 14 polygynous, 1 queenless). Two colonies in this subset had produced only female sexuals (range 19 to 59) and four only male sexuals (range 15 to 23). Genetic analysis of a total of 262 workers (10-20 workers per colony) revealed considerable variability at both investigated loci (L18: five alleles, H_{exp}=0.709; LXGT 223: nine alleles, H_{exp}=0.819). The mean inbreeding coefficient averaged over both loci was 0.020±0.045 (SE), suggesting random mating. The average intracolony relatedness among workers was 0.516±0.051. Polygynous and monogynous colonies did not differ in their average within-colony worker relatedness (5 monogynous colonies, r=0.578±0.108; 14 polygynous colonies, r=0.493±0.057; two-sided t-test, df=18, t=1.13, $P>0.10$). The power of this test was rather low (β=0.65).

The sex ratio in these colonies was again neither correlated with queen number, colony size, nor total sexual production (Table. 1). However, it was significantly correlated with worker relatedness (Table 1). The 8 colonies with a worker relatedness below 0.5 produced a considerably less female-biased sex ratio than the 12 colonies with a relatedness above 0.5 (Mann-Whitney U-test, U=24, P=0.064; Fig. 2).
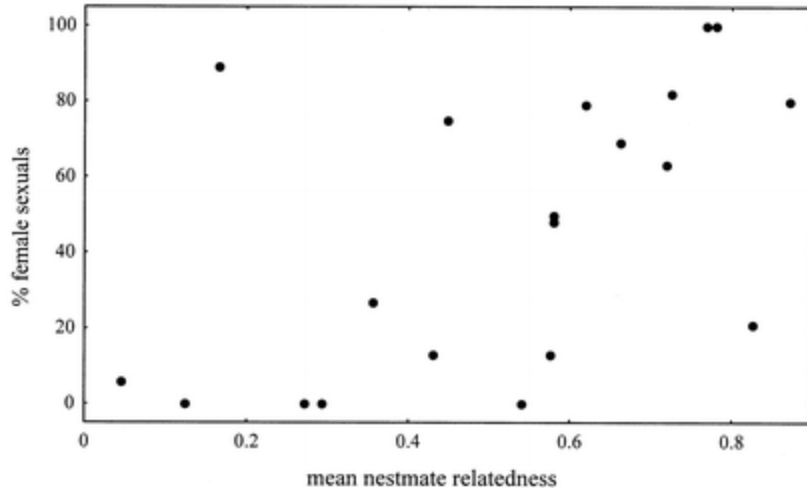
Fig. 2. Relationship between average within-colony worker relatedness and proportional investment in female sexuals in colonies of the ant *L. acervorum*

Discussion

In summer 1998, the population-wide sex ratio in the Reichswald population of the facultatively polygynous, monandrous ant, *L. acervorum*, was found to be quite balanced, with almost equal numbers of male and female sexuals produced. This overall value is quite close to ratios previously observed in the Santon population in England (Chan and Bourke *1994*; Chan et al. *1999*) and fits the expectation for polygynous ants. Total sexual production was positively correlated with colony size and negatively with queen number. Because the production of new workers was not monitored, we do not know whether this is caused by colonies with a larger queen-worker ratio switching to the production of workers or simply because they are less productive.

Sex ratios differed considerably between colonies and were highly split among all colonies. This result may in part be due to chance effects in less-productive colonies, but sex ratios are bimodally distributed even when considering only colonies that produced more than ten sexuals each. Colony-level proportional investment in female sexuals was neither correlated with colony size nor total sexual production.

Productivity presumably depends on the colony's resource level (e.g., Chan et al. *1999*). Consequently, the lack of correlation between sex ratio and total sexual production might indicate that sex allocation does not vary with resource availability, in contrast to predictions from the multifaceted parental-investment hypothesis (Rosenheim et al. *1996*), the cost variation hypothesis (Nonacs *1986*), the constant-male and the constant-female hypothesis (Frank *1987*; for reviews see also Bourke and Franks *1995*; Chan et al. *1999*; Chapuisat and Keller *1999*). Polygynous colonies reared on average more male biased sexual broods than monogynous colonies, but this difference was not significant. The number of queens present in the colony therefore appears to have a rather weak effect on sex ratio, if any. This stands in striking contrast to observations in the Santon population (Chan and Bourke *1994*; Chan et al. *1999*), but matches data from other British populations (Chan et al. *1999*) and *Myrmica tahoensis* (Evans *1995*). Our data therefore do not support the queen-worker conflict hypothesis (Herbers *1984*), which suggests that the queens' power to enforce their favorite sex allocation ratio increases with the

number of queens relative to the number of workers. The data also do not support the hypotheses that the value of female sexuals as replacement queens decreases at higher queen numbers (Brown and Keller *2000*) or that the queen-worker ratio is kept at an optimal level (O. Rüppell, J. Heinze, B. Hölldobler, unpublished data).

Nestmates in polygynous colonies that fragmented into colony buds were previously shown to be more closely related than nestmates in polygynous colonies that did not bud (Heinze et al. *1997b*). According to our present study, colonies with high relatedness produce a female-biased sex ratio (see below). Our data therefore also do not support the colony-budding hypothesis, according to which colonies that bud rear a more male-biased sex ratio (Boomsma *1993*). Sex ratios were clearly affected by the genetic structure of the colonies: nests with high worker relatedness produced predominantly female sexuals and colonies with low relatedness concentrated on the production of males. Assuming that, in *L. acervorum*, average worker relatedness is indicative of relatedness asymmetries (e.g., Chan et al. *1999*), this would meet predictions from the relatedness asymmetry hypothesis (Boomsma and Grafen *1990*, *1991*).

The life history of *L. acervorum*, with rapid changes in queen number (due to adoption, death, and budding) and a long duration of larval development (with sexual larvae hibernating at least once, often twice), results in a time lag between social and genetic colony structure (Heinze et al. *1995*; Bourke et al. *1997*; Chan et al. *1999*). In such cases, worker relatedness might occasionally better reflect relatedness asymmetries than the number of queens present in the nest. For example, a monogynous colony might become polygynous through the adoption of female sexual offspring of the old queen. During the next summer, the now polygynous society will still contain workers and sexual brood which are predominantly progeny of the old queen. Though several fertile queens are now present, both worker relatedness and relatedness asymmetry are high. If the workers adaptively manipulated sex allocation not in the egg stage but during later larval or pupal development (as was shown by Chapuisat et al. *1997* in *Formica exsecta*), and if they used cues from the larvae or adult workers to determine the genetic structure of the society (Sundström *1994*; Evans *1995*; Sundström et al. *1996*; Chan et al. *1999*), the sex ratio resulting in this polygynous colony would be female biased. In contrast, several years later, the colony might have lost all but one queen through death or budding, but workers and sexuals would still be progeny of several related queens. Despite monogyny, worker relatedness and relatedness asymmetries would then be low, resulting in a male-biased sex ratio.

However, the adoption of unrelated queens and frequent changes in queen number, with workers and male and female sexuals being offspring of different queens, might weaken the assumed correlation between relatedness asymmetry and worker relatedness (Brown and Keller *2000*; Foitzik and Heinze *2000*; Heinze and Keller *2000*). Colonies of *L. acervorum* occasionally adopt alien queens (Stille and Stille *1992*), though the high average relatedness of nestmate queens in the Reichswald population (Heinze et al. *1995*) indicates that this does not occur very often. Nevertheless, to determine whether split sex ratios are adaptive to workers in our study population would require the exact determination of relatedness asymmetries, which as yet has not been possible because too few colonies produced sufficient numbers of both sexes.

In *L. acervorum*, the response of workers to the changing genetic structure of their colonies counteracts the "erosion" of worker relatedness: female sexuals, which are produced in colonies

with high relatedness, are adopted and produce offspring in these nests. The decrease in relatedness, which results from the production of offspring by numerous queens, leads to a male-biased sex investment ratio in the brood. This leads to a decline in queen number due to queen death and emigration, and because only one or a few remaining queens reproduce, the relatedness increases again. In analogy to the development of multicellular organisms from a single fertilized cell, which has been considered to minimize between-cell conflict (Maynard Smith and Szathmáry *1995*), regular single-queen bottlenecks might help protect the colony against intraspecific social parasites concentrating on the production of sexuals.

References
Boomsma JJ (1993) Sex ratio variation in polygynous ants. In: Keller L (ed) Queen number and sociality in insects. Oxford University Press, Oxford, pp 86-109

Boomsma JJ, Grafen A (1990) Intraspecific variation in ant sex ratios and the Trivers-Hare hypothesis. Evolution 44:1026-1034

Boomsma JJ, Grafen A (1991) Colony-level sex ratio selection in eusocial Hymenoptera. J Evol Biol 3:383-407

Bourke AFG (1991) Queen behaviour, reproduction and egg cannibalism in multiple-queen colonies of the ant *Leptothorax acervorum*. Anim Behav 42:295-310

Bourke AFG, Franks (1995) Social evolution in ants. Princeton University Press, Princeton, NJ

Bourke AFG, Green HAA, Bruford MW (1997) Parentage, reproductive skew and queen turnover in a multiple-queen ant analysed with microsatellites. Proc R Soc Lond B 264:277-283

Brown WL, Keller L (2000) Colony sex ratios vary with queen number but not with relatedness asymmetry in the ant *Formica exsecta*. Proc R Soc Lond B 267:1751-1757

Bull JJ, Charnov EL (1988) How fundamental are Fisherian sex ratios? Oxf Surv Evol Biol 5:96-135

Chan GL, Bourke AFG (1994) Split sex ratios in a multiple-queen ant population. Proc R Soc Lond B 258:261-266

Chan GL, Hingle A, Bourke AFG (1999) Sex allocation in a facultatively polygynous ant: between-population and between-colony variation. Behav Ecol 10:409-421

Chapuisat M, Keller L (1999) Testing kin selection with sex allocation data in eusocial Hymenoptera. Heredity 82:473-478

Chapuisat M, Sundström L, Keller L (1997) Sex-ratio regulation: the economics of fratricide in ants. Proc R Soc Lond B 264:1255-1260

Crozier RH, Pamilo P (1996) Evolution of insect societies. Oxford University Press, Oxford

Evans JD (1995) Relatedness threshold for the production of female sexuals in colonies of a polygynous ant, *Myrmica tahoensis*, as revealed by microsatellite DNA analysis. Proc Natl Acad Sci USA 92:6514-6517

Fisher RA (1930) The genetical theory of natural selection. Clarendon, Oxford

Foitzik S, Heinze J (2000) Intraspecific parasitism and split sex ratios in a monogynous and monandrous ant. Behav Ecol Sociobiol 47:424-431

Foitzik S, Haberl M, Gadau J, Heinze J (1997) Mating frequency of *Leptothorax nylanderi* ant queens determined by microsatellite analysis. Insectes Soc 44:219-27

Frank SA (1987) Variable sex ratio among colonies of ants. Behav Ecol Sociobiol 20:195-201

Hamaguchi K, Ito Y, Takenaka O (1993) GT dinucleotide repeat polymorphisms in a polygynous ant, *Leptothorax spinosior* Forel and their use for measurement of relatedness. Naturwissenschaften 80:179-181

Heinze J, Keller L (2000) Alternative reproductive strategies: a queen perspective in ants. Trends Ecol Evol 15:508-512

Heinze J, Ortius D (1991) Social organization of *Leptothorax acervorum* from Alaska (Hymenoptera: Formicidae) Psyche 98:227-240

Heinze J, Lipski N, Schlehmeyer K, Hölldobler B (1995) Colony structure and reproduction in the ant, *Leptothorax acervorum*. Behav Ecol 6:359-367

Heinze J, Puchinger W, Hölldobler B (1997a) Worker reproduction and social hierarchies in *Leptothorax* ants. Anim Behav 54:849-864

Heinze J, Elsishans C, Hölldobler B (1997b) Sociogenetic consequences of budding in the ant, *Leptothorax acervorum*. Naturwissenschaften 84:249-250

Herbers JM (1984) Queen worker conflict and eusocial evolution in a polygonous ant species. Evolution 38:631-643

James FC, McCulloch CE (1990) Multivariate analysis in ecology and systematics: panacea or Pandora's box? Annu Rev Ecol Syst 21:129-166

Komdeur J, Daan S, Tinbergen J, Mateman C (1997) Extreme adaptive modification in sex ratio of the Seychelles warbler's eggs. Nature 385:522-525

Manly BFJ (1997) Randomization, bootstrap and Monte Carlo methods in biology, 2nd edn. Chapman & Hall, London

Maynard Smith J, Szathmáry E (1995) The major transitions in evolution. Freeman, Oxford

Nonacs P (1986) Ant reproductive strategies and sex allocation theory. Q Rev Biol 61:1-21

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258-275

Rosenheim JA, Nonacs P, Mangel M (1996) Sex ratios and multifaceted parental investment. Am Nat 148:501-535

Stille M, Stille B (1992) Intra- and inter-nest variation in mitochondrial DNA in the polygynous ant *Leptothorax acervorum* (Hymenoptera; Formicidae). Insectes Soc 39:335-340

Sundström L (1994) Sex ratio bias, relatedness asymmetry and queen mating frequency in ants. Nature 367:266-268

Sundström L, Chapuisat M, Keller L (1996) Conditional manipulation of sex ratios by ant workers: a test of kin selection theory. Science 274:993-995

Thorén P, Paxton R, Estoup A (1995) Unusually high frequency of $(CT)_n$ and $(GT)_n$ microsatellite loci in a yellowjacket wasp, *Vespula rufa* (L.) (Hymenoptera: Vespidae). Insect Mol Biol 4:141-148

Trivers RL, Hare H (1976) Haplodiploidy and the evolution of the social insects. Science 191:249-263