

QUEEN POLYMORPHISM IN *LEPTOTHORAX SPEC. A* :
ITS GENETIC AND ECOLOGICAL BACKGROUND
(HYMENOPTERA : FORMICIDAE)

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SUMMARY

Queen polymorphism in the non-parasitic ant, *Leptothorax spec. A*, is most probably genetically mediated by a pair of alleles E/e. E suppresses the development of wings, thoracic structures and ocelli in female larvae. Only ee-larvae may grow to gynomorphic, winged queens, EE- and Ee- larvae develop into intermorphic, wingless queens. The frequency of intermorphic queens varies widely in different habitats. Whereas in homogeneous coniferous forests throughout southern and central Quebec the gynomorph is abundant, in patchily distributed rocky outcrops along the St. Lawrence River intermorphic queens predominate. We suggest a different dispersal success of the two morphs in the various habitats.

ZUSAMMENFASSUNG

**Königinnen-Polymorphismus bei *Leptothorax spec. A* :
Genetischer und ökologischer Hintergrund (Hymenoptera - Formicidae)**

Der Königinnenpolymorphismus der nicht-parasitischen Ameisenart *Leptothorax spec. A* wird genetisch, wahrscheinlich durch ein Allelpaar E/e, kontrolliert. E unterdrückt dabei in weiblichen Larven die Entwicklung von Flügeln, Ocellen und Thoraxnähten, nur ee-Larven können zu geflügelten, gynomorphen Jungköniginnen heranwachsen. EE- und Ee-Königinnen sind immer intermorph. Die Häufigkeit intermorpher Königinnen im Freiland variiert von Population zu Population: in den homogenen, lichten Nadelwäldern Süd/und Zentralquebecs überwiegt die Gynomorphe, in inselartig beschränkten Biotopen, wie auf den Felsflächen am Lorenzstrom, dominieren intermorpher Weibchen. Unterschiedlicher Erfolg der beiden Morphen in den verschiedenen Biotopen wird angenommen.

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INTRODUCTION

The typical ant queen is morphologically distinct from the worker. Queens (♀♀) are gynomorphic, they usually have ocelli, they have wings, which are shed after mating, and their voluminous thorax consists of individual sclerites. The workers (♂♂), on the other hand, are ergatomorphic, they never have wings and the thoracic sclerites are widely fused. In the ant subfamily Myrmicinae, ergatomorphs usually don't have ocelli.

In a number of species, however, queens are regularly found, which are workerlike or intermorphic, i.e. showing morphologically features intermediate between those of gynomorphs and ergatomorphs. In intermorphic queens, wings and ocelli are reduced and their thoracic structures are simplified.

Intermorphic and ergatomorphic queens are known from several species of ants belonging to the more primitive subfamilies Ponerinae or Cerapachyinae. Among the higher ants they occur in some social parasitic species, such as *Polyergus rufescens*, *Tetramorium ergatogyna*, *Harpagoxenus sublaevis*, the guest-ant genus *Formicoxenus*, *Leptothorax wilsoni*, and *Aporomyrmex ampeloni*.

Queen polymorphism, the coexistence of several morphologically different queens within one species, in non-parasitic higher ants is known only from a couple of species. In the literature, intermorphic or ergatomorphic queens are listed, e.g., for several *Monomorium* (BOLTON 1986, DU BOIS 1986), and two North American species of the genus *Leptothorax* (FRANCOEUR 1986, HEINZE and BUSCHINGER 1987). Recently we have studied the morphology of intermorphic and gynomorphic queens in one of the latter species. This non-parasitic ant belongs to the North American *Leptothorax "muscorum"* complex (BROWN 1955) and is not yet formally described. Therefore, we here again refer to it as *Leptothorax spec. A*.

L. spec. A is a common ant in the boreal forests of Northeast America. The frequency of colonies with intermorphic queens varies widely from population to population. In Quebec it ranges from about 15 % in Grands Jardins National Park to over 80 % in Tadoussac.

Queen polymorphism in ants has been thoroughly studied only in the palaeartic slave-maker ant, *Harpagoxenus sublaevis*. Here the queen morph is most probably genetically mediated by one single, diallelic locus (BUSCHINGER 1975, 1978). The dominant allele E suppresses the development of wings, thoracic sclerites and ocelli in larvae, which are epigenetically determined to become queens. EE — and Ee — queens always are intermorphic. Only from ee-brood are gynomorphic, winged females reared. If a gynomorph mates with the son of a homozygous intermorphic queen, the young queens in her offspring will be intermorphic females (ee x E → Ee). The alleles do not affect the reproductive organs. Intermorphs and gynomorphs both

have eight ovarioles and a receptacle, they both mate and may become fertile queens. Ergatomorphic workers usually have simplified ovaries, consisting of two to eight ovarioles, and lacking the receptacle (BUSCHINGER and WINTER 1978). In some cases ergatomorphic workers and intermorphic queens could only be distinguished by dissection.

The genotypes EE, Ee, and ee have strong influence on the ratio of workers and young queens reared in the colonies. In four breeding cycles, colonies of the mating types $EE \times E$ and $ee \times e$ produced almost equal numbers of female descendants, but the ♀♀/♂♂ ratio differed significantly. In total, 18.8 % of the homozygous EE — and 60.3% of the ee-brood became young queens. Caste determination in *H. sublaevis* is influenced by the E/e-system in two ways: the E-allele slows down the development of the brood, and the inhibitory capacity of EE-queens is higher than that of ee-queens (WINTER and BUSCHINGER 1986).

Since in about 99 % of all colonies collected from the field the queen was an intermorph, there is apparently a strong ecological advantage for wingless females. Two factors favor intermorphic queens. One is the higher worker-bias. The more ♂♂ are reared in a colony of the slave-maker *Harpagoxenus*, the more successful are the slave-raids. Second, host-species colonies occur in highly variable densities, and suitable sites with high population densities are patchily distributed. An intermorphic young queen, thus, may have a better chance to find a host nest for colony foundation in the vicinity of her mother's nest than an alate, gynomorphic queen who might drift off into unsuitable habitats during flight activity. Winged females, on the other hand, may be important for the infestation of new host populations (WINTER and BUSCHINGER 1986).

According to these authors, the best adapted colony type would be $Ee \times E$: Due to the caste-determining influence of E and e, these colonies produce a reasonably high number of ♂♂ from EE-larvae, and also many intermorphic queens from Ee-larvae. When the old queen dies after about 10 years the declining colony produces a very high number of males which then are worker-offspring. Since the workers are predominantly EE, their sons will be mostly E. In the population a high fraction of the young ergatoid females will be Ee (offspring from the queenright $Ee \times E$ colonies), and also a high fraction of the ♂♂ will be E (offspring from queenless colonies with laying EE-workers). Most young colonies then should be $Ee \times E$, again, and the system is balanced.

Almost nothing is known about the role of wingless queens in non-parasitic species. BOLTON (1986) developed the hypothesis, that in several species of *Monomorium* the loss of wings and other features of the gynomorph in the queen caste is a side-product of dependent colony foundation via polygyny and budding. The risk for a young queen is far lower, if she remains in her mother's colony after mating, becomes fertile there, and

finds a new colony by colony fission or budding, than during independent colony foundation. If resources are restricted, as on rocky islands or in deserts, colonies should prefer to invest energy into wingless females with a stationary sexual behavior, who are highly successful in colony foundation. Similarly, certain *Formica* species, whose queens are clumsy fliers and return to their mothers' nests, seem to have advantages in patchily distributed habitats (ROSENGREN and PAMILO 1983).

During the past few years we have extensively studied the biology of *Leptothorax spec. A* from various populations in New England. We here report results of experiments designed to ascertain whether the queen polymorphism of *Leptothorax spec. A* is genetically mediated, similar to that of *Harpagoxenus sublaevis*, and discuss the ecological background of queen polymorphism in this species.

MATERIAL AND METHODS

Complete colonies of *Leptothorax spec. A* and related species of the myrmicine tribe Leptothoracini were collected in 1979, 1983, 1985, 1987 and 1988 in Quebec, especially along Saguenay River and St. Lawrence River, and in New England. Laboratory colonies

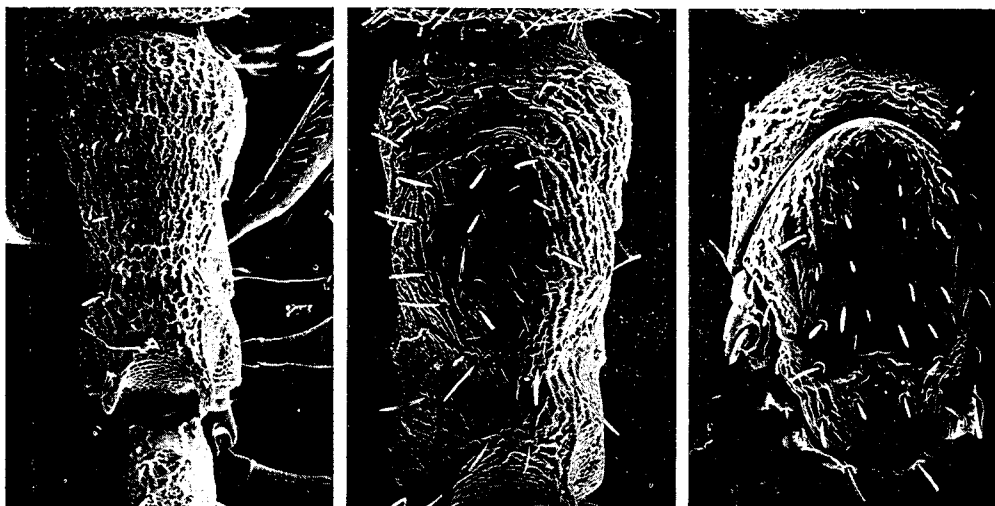


Fig. 1. — Thorax of an ergatomorphic worker, an intermorphic queen and a gynomorphic queen of *Leptothorax spec. A* (from left to right). The development of thoracic sutures in intermorphic females varies widely. The picture shows a female with a nearly intermediate morphology. Pictures were taken by A. Maiazza, using the scanning electron microscope of the FB Geologie, TH Darmstadt.

Abb. 1. — Thorax einer ergatomorphen Arbeiterin, einer intermorphen und einer gynomorphen Königin von *Leptothorax spec. A* (von links nach rechts). Die Thoraxstruktur intermorpher Weibchen ist recht variabel, dargestellt ist eine Intermorphe mit intermediärer Thoraxentwicklung. REM-Aufnahmen durch A. Maiazza, FB Geologie, TH Darmstadt.

were kept for several artificial breeding cycles as described by BUSCHINGER (1974). Since *Leptothorax spec. A* shows a stationary sexual calling behavior ("Locksterzeln", BUSCHINGER 1968), it was easy to mate sexuals of defined origin in the laboratory.

Females and workers were dissected according to the method described by BUSCHINGER and ALLOWAY (1978).

Conspecificity of colonies from different habitats was proven using karyological techniques (IMAI *et al.* 1977) and enzyme gel electrophoresis, as morphological features vary widely in this group of *Leptothorax*.

Esterase, MDH and other enzymes were separated by isoelectric focusing of crude pupal homogenates in ultrathin polyacrylamide gels (HEINZE and BUSCHINGER 1988).

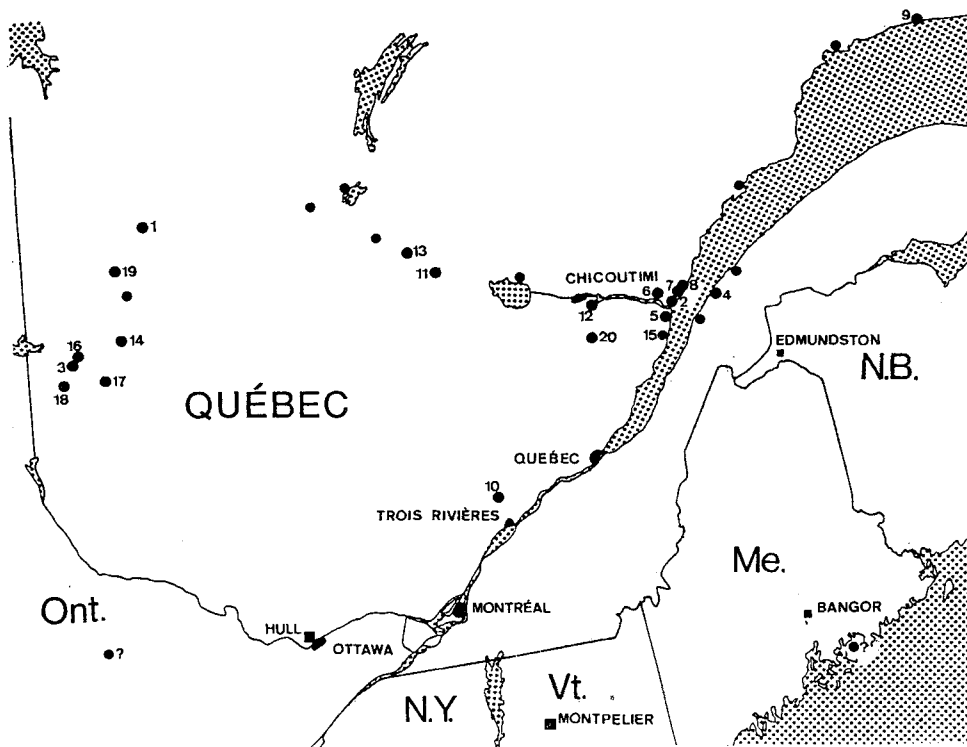


Fig. 2. — Collecting sites of *Leptothorax spec. A*. Numbers correspond to those given in table I. In unnumbered sites less than three queenright colonies each have been collected. Dots with a question mark indicate collecting sites of colonies of whose conspecificity we are not yet sure (Ontario and Mt. Desert Island, Me.).

Abb. 2. — Fundpunkte von *Leptothorax spec. A*. Die Nummern entsprechen denen in Tabelle I. Weitere Fundstellen, an denen weniger als drei Kolonien mit Königin gesammelt werden konnten, sind ohne Nummer eingezeichnet. Fragezeichen (in Ontario und Mt. Desert Island, Me.) stehen für Kolonien zweifelhafter Artzugehörigkeit.

RESULTS

Leptothorax spec. A has a queen-polymorphism with gynomorphic (G ♀ ♀) and intermorphic females (I ♀ ♀), which both may be fully fertile queens in the field. Intermorphs differ from ergatomorphic workers in the presence of at least one minute ocellus and in most cases of some more or less distinct thoracic sutures (fig. 1). In contrast to ergatomorphs and occasional intergrades between ♀ ♀ and ♂ ♂ in other species of *Leptothorax*, I ♀ ♀ of *L. spec. A* always have a fully developed spermatheca.

The frequency of queen morphs varies widely in the field (table 1).

Table I. — Frequency of colonies with gynomorphic females in different populations. Biotopes are characterized by the letters A to D.

A: patchy, rocky outcrops, sparsely overgrown with lichen, American laurel, blueberries, few bushes and young trees, surrounded by dense thickets or forests.
 B: extended, though patchy, rocky areas, surrounded by light coniferous forests.
 C: homogeneous, light forests, mostly pines or spruces.
 D: open homogeneous shrub forests with young or dwarfed birches and alders.
 The geographical location of the populations is indicated in fig. 2. Site 21, Mt. Monadnock in New Hampshire, is not shown on the map.

Tabelle I. — Häufigkeit von Kolonien mit gynomorphen ♀ ♀ in verschiedenen Populationen. Biotope sind durch die Buchstaben A bis D charakterisiert.

A: isolierte, von Flechten, *Kalmia*, Blaubeeren, einigen Büschen und jungen Bäumen spärlich bewachsene Felsflächen inmitten dichter Gebüsch oder Wälder.
 B: ausgedehnte, isolierte Felsflächen in lichterem Nadelwäldern.
 C: homogene, lichte Wälder, meist Kiefern oder Fichten.
 D: offene Gebüsch mit jungen oder zwergwüchsigen Birken und Erlen.
 Die Lage der verschiedenen Populationen ist Fig. 2 zu entnehmen. Fundpunkt 21, Mt. Monadnock in New Hampshire, ist nicht eingezeichnet.

| Population | Biotope | n col. | % G ♀ ♀ |
|----------------------------|---------|--------|---------|
| 1 Matagami | A | 14 | 7 |
| 2 Tadoussac | A | 145 | 18 |
| 3 Lac La Haie, | B | 5 | 20 |
| 4 Cap à l'Original | A | 4 | 25 |
| 5 Baie Sainte-Catherine | A | 3 | 33 |
| 6 Sacré-Cœur du Saguenay | C | 3 | 33 |
| 7 Grandes-Bergeronnes | A | 6 | 33 |
| 8 Les Escoumins | C | 3 | 33 |
| 9 Rivière Romaine | A | 9 | 33 |
| 10 Shawinigan | A | 3 | 33 |
| 11 Rivière Cran | B | 11 | 36 |
| 12 La Baie | A | 35 | 40 |
| 13 Rivière Vermillon | B | 5 | 40 |
| 14 Saint-Félix de Dalquier | A | 5 | 40 |
| 15 Saint-Siméon | C | 24 | 54 |
| 16 Lac du Sablon | B | 7 | 57 |
| 17 Cadillac | B | 6 | 66 |
| 18 Rouyn-Noranda | B | 7 | 71 |
| 19 route 109, km 170 | C | 9 | 78 |
| 20 Grands Jardins | C | 28 | 88 |
| 21 Mount Monadnock, N.H. | D | 34 | 97 |

In patchily distributed areas, like on the rocky outcrops along the St. Lawrence River in Tadoussac or in Matagami, I♀♀ by far outnumber gynomorphs. G♀♀ are abundant in homogeneous habitats, light coniferous forests such as in St. Siméon or Grands Jardins, or open scrubby forests such as on Mt. Monadnock.

53 colonies of *Leptothorax spec. A* from different populations were kept in the laboratory for up to ten breeding cycles (about five years), where they regularly produced sexual offspring and workers. The morphs of the female sexuals remained constant in each colony throughout this time. In addition to males and ergotomorphic workers,

- 9 gynomorphic queens produced only gynomorphic offspring,
- 4 gynomorphic queens produced only intermorphs,
- 8 intermorphic queens produced both morphs, and
- 32 intermorphic queens produced only intermorphic females.

No gynomorph produced both morphs, and no intermorph produced only gynomorphic offspring.

These observations indicate a genetic influence on morph determination, similar to that in *Harpagaxenus sublaevis*, with a dominant allele E affecting the development of ocelli, wings and thoracic structures in the female brood. According to this hypothesis, gynomorphs should always be ee. Their sons therefore, should carry the allele e. Intermorphic females, however, may be Ee and EE, and morphologically there is no difference between females of the latter genotypes. The sons of intermorphic queens therefore may be E or e. Cross-breeding experiments of mating types 2, 4 5 and 6 (*table III*) thus may lead to different results. If, e.g., an intermorphic female of unknown genotype mates with the son of an intermorphic queen, four different mating types are possible: EE × E, EE × e, Ee × E and Ee × e.

By choice of males and females from certain populations we devised our experiments in a way that one of the possible mating types became more probable than others.

If both the morph of the queen and that of her daughters are known, in some cases the hypothetical mating type can be deduced. It should be

ee × e in colonies with a gynomorphic queen, who produces gynomorphic females only,

ee × E in colonies with a gynomorphic queen, who produces intermorphic offspring, and

Ee × e in colonies with an intermorphic queen, who produces both gynomorphic and intermorphic females.

The solutions of the cubic equation.

$$p^3 (EE \times E) + p^2q (EE \times e) + pq^2 (ee \times E) + 2p^2q (Ee \times E) + 2pq^2 (Ee \times e) + q^3 (ee \times e) = 1.$$

(where p and q are the frequencies of the alleles E and e) with the

observed frequencies of colonies with known mating type in the extensively studied population of Tadoussac give estimated values for p and q (table II). With respect to the queen morph, the population seems not to be in a Hardy-Weinberg equilibrium; allele-frequencies vary widely according to the data set used as basis for the evaluation. Frequencies of $p = 0.85$ and $q = 0.15$ seem to be closest to the observed conditions in the field.

Thus, most of the intermorphic females in the population of Tadoussac should be homozygous, EE . Males, produced by them, should carry the allele E . These males were used in cross-breeding experiments of type 2, 4, and 6 (table III). Intermorphic females, mated in experiments 5 and 6, also were reared in colonies from populations, in which intermorphs far outnumbered gynomorphic queens. The intermorphic females used in breeding experiments 3 and 4, were either offspring of a gynomorphic queen or of an intermorphic female, who produced both female morphs. In both cases, intermorphic females should be heterozygous: Ee . The hypothesis presupposes, however, that colonies are monogynous and that queens mate only once. In previous studies we have demonstrated by dissection functional monogyny in *Leptothorax spec. A*; in each colony only one single female is fertile. Other females, though often inseminated, remain sterile (HEINZE and BUSCHINGER, 1987). In the laboratory, young females stopped sexual calling behavior after one successful copulation with a male. Electrophoretic studies gave further evidence for single mating in the field. Esterase 7 is variable in most species of the subgenus *Leptothorax* s.str., and in *L. spec. A* at least four different allozymes can be separated by isoelectric focusing (HEINZE 1987). We screened Est-7 patterns in five to ten pupae each from 36 colonies, all collected from the population in Tadoussac. The allozymes were found in most possible heterozygous and homozygous combinations.

Multiple mating in some cases should result in more than two different allozyme combinations per colony, e.g. $AB \times C/D \rightarrow AC, BC, AD, CD$. We never found more than two different patterns per colony. The frequency of homozygous genotypes apparently is somewhat increased; all pupae in 11 of 36 colonies showed the same single band, suggesting a homozygous genotype throughout the colony. The results, however, do not indicate strong inbreeding in the population in Tadoussac.

Out of 175 ♀♀, mated with ♂♂ in the laboratory, 68 have produced female sexual offspring for three, four, or even five breeding cycles. With the exception of one colony, where in addition to 47 ♀♀ and 38 gynomorphic ♀♀ four wingless ♀♀ were reared, all gynomorphic queens, mated with the son of a gynomorph, produced gynomorphic offspring ($ee \times e \rightarrow ee$, # 1 in table III). Intermorphic ♀♀ arose in cross-breedings of gynomorphic ♀♀ with the sons of intermorphic queens ($ee \times E \rightarrow Ee$, # 2, in table III). Both morphs were produced by heterozygous intermorphic

Table II. — Estimated frequencies p and q of the hypothetical alleles E and e in the Tadoussac population, based on observed frequencies of different colony types in the field. The colony types used as the basis for the calculation were $ee \times e$ — colonies (gynomorphic queens producing gynomorphic offspring only) in 1, $Ee \times e$ — colonies (intermorphic queens producing both morphs) in 2, and the ratio of $ee \times E$ and $ee \times e$ — colonies in 3.

Tabelle II. — Anteile p und q der hypothetischen Allele E und e in der Population Tadoussac, berechnet aus den beobachteten Häufigkeiten verschiedener Kolonietypen im Freiland. Grundlage der Berechnung waren $ee \times e$ — Kolonien (gynomorphe Königin, die gynomorphe Jungweibchen produziert) in 1, $Ee \times e$ — Kolonien (intermorphe Königin produziert beide Morphen) in 2 und das Verhältnis von $ee \times E$ zu $ee \times e$ — Kolonien in 3.

| | Frequencies of different colony types | | | Observed |
|---------------|---------------------------------------|------|------|----------|
| | 1 | 2 | 3 | |
| $EE \times E$ | 0.18 | 0.61 | 0.02 | ? |
| $EE \times e$ | 0.14 | 0.11 | 0.05 | ? |
| $Ee \times E$ | 0.28 | 0.22 | 0.11 | ? |
| $Ee \times e$ | 0.21 | 0.04 | 0.25 | 0.04 |
| $ee \times E$ | 0.11 | 0.02 | 0.14 | 0.03 |
| $ee \times e$ | 0.08 | 0.00 | 0.39 | 0.08 |
| pE | 0.57 | 0.85 | 0.27 | ? |
| qe | 0.43 | 0.15 | 0.73 | ? |

queens, which had been mated with an e -male ($Ee \times e \rightarrow Ee + ee$, # 3 in table III).

In cross-breeding experiments of the types 4, 5, and 6, with the exception of two $G \text{♀} \text{♀}$ in one colony, only intermorphs were reared as female sexual offspring.

In most matings, the colonies of males and females came from different populations. After mating with males from Tadoussac, e.g., $G \text{♀} \text{♀}$ from populations with a high frequency of gynomorphs reared intermorphs as did $G \text{♀} \text{♀}$ from Tadoussac.

One additional experiment provides further evidence for a genetic control of queen polymorphism. In all populations known *Leptothorax spec. A* lives sympatrically with a second undescribed species, *Leptothorax spec. B*. Both taxa are easily distinguished by karyo-type, colony structure, and MDH- or IDH-isozymes (HEINZE and BUSCHINGER 1987, HEINZE 1987), and also differ morphologically. In all of 222 colonies of *L. spec. B* the queen was gynomorphic. In the field, hybrids apparently are very rare. Isolation is guaranteed by different times of day of sexual activity. In the laboratory, however, we successfully mated a gynomorphic female of *spec. B* with a son of an intermorphic *spec. A*-female. In four breeding cycles, the gynomorphic *spec. B*-female produced workers and young queens: all 17 female sexuals were intermorphs. All hybrid $I \text{♀} \text{♀}$ had six ovarioles and a receptacle. Hybridization has been verified by comparison of MDH-patterns and by preparation of chromosomes from the brains of female prepupae. Hybrids show MDH-isozymes from both parental

Table III. — Queen morphs obtained in the crossbreeding experiments with *Leptothorax spec. A*. $G\varnothing\varnothing$ and $I\varnothing\varnothing$ are gynomorphic and intermorphic females, $G\delta\delta$ and $I\delta\delta$ males produced by gynomorphic or intermorphic queens, respectively. The most probable genotypes of the parents (see text) are underlined.

Tabelle III. — Ergebnisse der Kreuzungsexperimente mit *Leptothorax spec. A*. $G\varnothing\varnothing$ und $I\varnothing\varnothing$ steht dabei für gynomorphe bzw. intermorphe Weibchen, $G\delta\delta$ und $I\delta\delta$ sind Männchen, die von gynomorphen bzw. intermorphen Weibchen produziert wurden. Der wahrscheinlichste Genotyp der Kreuzung ist jeweils unterstrichen (vergl. Text).

| # | Mated sexuals | Possible genotype | Female morphs | | n. col. |
|--------|-------------------------------|----------------------|---|---|---------|
| | | | Expected | Observed | |
| ser. 1 | $G\varnothing \times G\delta$ | <u>ee</u> \times e | $G\varnothing\varnothing$ | 108 $G\varnothing\varnothing$ 4 $I\varnothing\varnothing$ * | 10 |
| ser. 2 | $G\varnothing \times I\delta$ | <u>ee</u> \times E | $I\varnothing\varnothing$ | 0 $G\varnothing\varnothing$ 210 $I\varnothing\varnothing$ | 20 |
| | | ee \times e | $G\varnothing\varnothing$ | | |
| ser. 3 | $I\varnothing \times G\delta$ | <u>Ee</u> \times e | $G\varnothing\varnothing + I\varnothing\varnothing$ | 46 $G\varnothing\varnothing$ 229 $I\varnothing\varnothing$ | 11 |
| ser. 4 | $I\varnothing \times I\delta$ | <u>Ee</u> \times E | $I\varnothing\varnothing$ | 0 $G\varnothing\varnothing$ 393 $I\varnothing\varnothing$ | 11 |
| | | Ee \times e | $G\varnothing\varnothing + I\varnothing\varnothing$ | | |
| ser. 5 | $I\varnothing \times G\delta$ | <u>EE</u> \times e | $I\varnothing\varnothing$ | 0 $G\varnothing\varnothing$ 10 $I\varnothing\varnothing$ | 1 |
| | | Ee \times e | $G\varnothing\varnothing + I\varnothing\varnothing$ | | |
| ser. 6 | $I\varnothing \times I\delta$ | <u>EE</u> \times E | $I\varnothing\varnothing$ | 2 $G\varnothing\varnothing$ 228 $I\varnothing\varnothing$ | 8 |
| | | EE \times e | $I\varnothing\varnothing$ | | |
| | | Ee \times E | $I\varnothing\varnothing$ | | |
| | | Ee \times e | $G\varnothing\varnothing + I\varnothing\varnothing$ | | |

* The four $I\varnothing\varnothing$ were reared in one single colony.

species. Whereas *Leptothorax spec. A* typically has $2n = 30$ chromosomes and *spec. B* $2n = 36$, we counted 60 or more chromosomes in metaphase plates of the hybrid.

In cross-breeding of the mating type $Ee \times e$, # 3 in table III, both morphs should be produced in equal numbers. Ratios of $I\varnothing\varnothing$ and $G\varnothing\varnothing$ in our experiments, however, differ widely from a 1:1-segregation. They vary from colony to colony and range from 0 $G\varnothing\varnothing$ /12 $I\varnothing\varnothing$ to 14 $G\varnothing\varnothing$ /7 $I\varnothing\varnothing$ (table IV). A similar variability of ratios was also observed in colonies from the field. There the production of $G\varnothing\varnothing$ not only differs between colonies, but also within a single colony from year to year. In all, most colonies produced more $I\varnothing\varnothing$ than $G\varnothing\varnothing$.

A first census of $\varnothing\varnothing$ in colonies of defined genotype suggests an influence of the alleles E/e on caste-determination in *Leptothorax spec. A*, like in *H. sublaevis*, though weaker. In colonies of the hypothetical mating-type $EE \times E$ during three breeding-cycles, 20.9 percent of the female brood eclosed to sexuals, compared to 44.1 percent in the $ee \times e$ — colonies. Offspring were counted only in colonies that were still queenright after the third breeding-cycle (table V). Differences in brood production are highly significant ($p < 0.001$, G-test).

Recently we have shown (HEINZE and BUSCHINGER 1987) that $G\varnothing\varnothing$ are significantly larger than $I\varnothing\varnothing$. To assess an influence of the hypothetical alleles E/e on workers and males, thorax length of individuals produced

Table IV. — Production of intermorphic and gynomorphic ♀♀ in Ee × e — colonies.

Tabelle IV. — Produktion von intermorphen und gynomorphen ♀♀ in Kolonien des Kreuzungsschemas Ee × e.

| Colony | G ♀♀ | I ♀♀ | ♂♂ | ratio G ♀♀/(I ♀♀ + G ♀♀) |
|--------|------|------|-----|--------------------------|
| # 11 | 8 | 27 | 190 | 0.23 |
| # 46 | 2 | 67 | 252 | 0.03 |
| # 51 | 4 | 69 | 196 | 0.05 |
| # 64 | 0 | 7 | 33 | 0.00 |
| # 97 | 0 | 1 | 9 | 0.00 |
| # 107 | 14 | 7 | 19 | 0.67 |
| # 111 | 1 | 1 | 2 | 0.50 |
| # 125 | 12 | 10 | 11 | 0.55 |
| # 194 | 4 | 23 | 24 | 0.15 |
| # 202 | 0 | 12 | 59 | 0.00 |
| # 213 | 1 | 5 | 6 | 0.17 |
| total | 46 | 229 | 801 | 0.17 |

Table V. — Ratio of ♀♀/♂♂ produced in the offspring of EE × E — and ee × e — colonies.

Differences between ♀♀/♂♂ ratios are significant, $p < 0.001$, 2×2 test of independence (G-Test).

Tabelle V. — Produktionsverhältnis ♀♀/♂♂ in der Nachkommenschaft von EE × E — und ee × e — Kolonien.

Die Unterschiede im Produktionsverhältnis sind nach dem G-Test signifikant ($p < 0.001$).

| Colony | Hypothetical mating type | ♀♀ | ♂♂ | % ♀♀ |
|--------|--------------------------|-----|-----|------|
| # 98 | ee × e | 42 | 47 | 47.2 |
| # 99 | ee × e | 10 | 14 | 41.7 |
| # 114 | ee × e | 40 | 27 | 59.7 |
| # 115 | ee × e | 23 | 31 | 42.6 |
| # 118 | ee × e | 19 | 54 | 26.0 |
| # 195 | ee × e | 30 | 35 | 46.1 |
| | total | 164 | 208 | 44.1 |
| # 8 | EE × E | 45 | 116 | 27.9 |
| # 16 | EE × E | 18 | 124 | 12.7 |
| # 18 | EE × E | 19 | 70 | 21.3 |
| | total | 82 | 310 | 20.9 |

either by a gynomorphic or an intermorphic mother was measured (table VI). Sons of a gynomorphic queen are significantly larger than sons of intermorphic females, and the same tendency was found in ♂♂ from most populations. Differences in worker size are quite small and data were not normally distributed. Colonies with gynomorphic queen usually had a number of very small workers, who sometimes were even smaller than smallest workers in colonies with intermorphic queen. The average ♂♂ in G♀ — colonies, however, are larger than average ♂♂ in colonies with I♀♀.

Table VI. — Thorax length (TL) of ♀♀, ♂♂, and ♂♂ of *Leptothorax spec. A* from colonies with gynomorphic or intermorphic queen.

Significance of differences in thorax size were usually tested with t- and U-test. Since TL of workers in most populations was not normally distributed, the test by Smirnof-Kolmogoroff was applied in these cases.

Tabelle VI. — Thoraxlänge (TL) von ♀♀, ♂♂ und ♂♂ Kolonien von *Leptothorax spec. A* mit gynomorpher bzw. intermorpher Königin.

Auf Signifikanz der Unterschiede in der Thoraxlänge wurde mit t- und U-Test geprüft, im Falle der ♂♂ auch mit dem Test für nicht normal-verteilte Werte nach Smirnof-Kolmogoroff.

| | Colonies with gynomorphic queen | | Colonies with intermorphic queen | | Difference | |
|---|---------------------------------|---------------|----------------------------------|---------------|------------|----------|
| | n | TL [mm] | n | TL [mm] | [mm] | |
| ♀♀ (data from all populations in Quebec) | 42 | 1.01 +/- 0.04 | 107 | 0.93 +/- 0.09 | 0.08 | p < 0.01 |
| ♂♂ (data from laboratory bred males) | 86 | 1.41 +/- 0.10 | 221 | 1.30 +/- 0.10 | 0.11 | p < 0.01 |
| ♂♂ (data from Tadoussac) | 80 | 0.90 +/- 0.05 | 120 | 0.89 +/- 0.04 | 0.01 | n.s. |
| ♂♂ (data from La Baie) | 91 | 0.89 +/- 0.05 | 120 | 0.86 +/- 0.04 | 0.03 | p < 0.01 |
| ♂♂ (data from Ashuapmushuan: colonies from Rivière Cran and Rivière Vermillon) | 92 | 0.94 +/- 0.05 | 109 | 0.88 +/- 0.07 | 0.06 | p < 0.01 |
| ♂♂ (data from Saint Félix de Dalquier) | 89 | 0.89 +/- 0.05 | 66 | 0.87 +/- 0.05 | 0.02 | p < 0.01 |
| ♂♂ (data from Grandes Bergeronnes) | 36 | 0.91 +/- 0.04 | 81 | 0.87 +/- 0.04 | 0.04 | p < 0.01 |

Dissection data show that ♂♂ from colonies with intermorphic females more often had ovaries with three, four five and six ovarioles than do ♀♀ from colonies with G♀♀ (table VII).

Table VII. — Percentage of workers with i ovarioles in different colony types. The most probable genotype of workers is indicated. Data are from colonies collected in the field and kept for four breeding cycles in the laboratory.

Differences in the frequencies of workers with more than two ovarioles are significant (p < 0.01), if colonies with intermorphic queen are compared to those with gynomorphic queen (G-Test).

Tabelle VII. — Häufigkeit von ♀♀ mit i Ovariolen in den verschiedenen Kolonietypen. Der wahrscheinlichste Genotyp der Arbeiterinnen ist angegeben. Präpariert wurden nur ♂♂ aus Freilandkolonien, die für vier Brutzyklen im Labor gehalten worden waren.

Der Unterschied zwischen der Häufigkeit von Arbeiterinnen mit mehr als zwei Ovariolen in Kolonien mit gynomorpher Königin und Kolonien mit intermorpher Königin ist gesichert (p < 0.01, G-Test).

| Colony type | Genotype | workers with i ovarioles [%] | | | | | |
|-----------------|----------|------------------------------|-------|-----|-----|-----|-----|
| | | n | i = 2 | 3 | 4 | 5 | 6 |
| Queen/offspring | | | | | | | |
| I♀ → I♀♀ | EE | 412 | 88.6 | 2.7 | 3.2 | 1.9 | 3.6 |
| G♀ → I♀♀ | Ee | 118 | 92.5 | 2.5 | 4.2 | 0.8 | — |
| I♀ → G♀♀ + I♀♀ | Ee/ee | 27 | 88.8 | 7.4 | 3.7 | — | — |
| G♀ → G♀♀ | ee | 98 | 97.0 | 1.5 | 1.5 | — | — |

DISCUSSION

Leptothorax spec. A is an unusual ant, being one of the few non-parasitic species of higher ants with several morphologically different types of queens. As in the slave-making leptothoracine *Harpagoxenus sublaevis*, queen morphs in *L. spec. A* are most probably genetically determined. Our cross-breeding results can be explained by the assumption of a single locus with two alleles E/e. E suppresses the development of thoracic structures, wings and ocelli in female larvae predetermined to become queens. Gynomorphs always are homozygous, ee, while intermorphs are EE and Ee. According to our one-locus-hypothesis, in colonies of the mating-type Ee × e both morphs of queens should be reared in equal amounts. In these colonies, however, the percentage of reared gynomorphs varies widely, and in total by far more intermorphs are produced.

We cannot exclude the possibility that two or more loci control queen morphs. More loci might better explain the segregation of G♀♀ and I♀♀ in cross-breeding experiments of the type Ee × e. If, e.g., a two-loci hypothesis is considered, in some colonies only 25 % gynomorphic offspring is expected, if gynomorphs are homozygous in two recessive alleles; if queen morphs are determined by three loci, some of the colonies should produce 12.5% gynomorphs and 87.5% intermorphs. A model with gynomorphs which are heterozygous in both loci, comparable to the caste-determining system proposed for stingless bees (KERR, 1950), would not explain why gynomorphic queens produce almost exclusively either intermorphic or gynomorphic offspring, but rarely both morphs.

If, however, external factors might influence a genetically mediated morph determination, as they apparently do in caste-determination in *Melipona* (KERR and NIELSEN, 1966; KERR *et al.*, 1966), our results can be explained with the E/e-hypothesis.

In *Harpagoxenus sublaevis*, in ee × e — colonies intermorphic females were regularly reared, too, suggesting, that some ee-larvae may develop into intermorphic females (BUSCHINGER 1978). The ratio of gynomorphic vs. intermorphic females in Ee × e — colonies of *Leptothorax spec. A* may be biased by the same effect, and the four intermorphs who were reared in one of our ee × e — colonies might be ee-intermorphs, too.

Though, following our assumption, a major part of the I♀♀ in colonies of series 3 should be of the genotype ee, only few ee-I♀♀ were produced in colonies of group 1. Winter and BUSCHINGER (1986) have shown that intermorphic queens of *Harpagoxenus sublaevis* have a stronger inhibitory influence on larvae than do gynomorphs. In colonies of the mating type EE × e far less intermorphic females and more ♂♂ were produced than in colonies of the type ee × E. The brood in both cases was of the same heterozygous genotype, Ee, but the morph of the queen differed. This

stronger inhibitory capacity of I♀♀ might not only explain the higher worker-bias in colonies with intermorphic queens (see below), but also the differences in the frequency of ee-intermorphs. In two or three field colonies that were recently collected, we found gynomorphic queens accompanied by seven or more gynomorphic females and one or two intermorphs. These I♀♀ perhaps were homozygous, ee.

The stronger inhibitory influence of *H. sublaevis* intermorphs on larvae is thought to be one of the reasons for the difference between the production of female castes by G♀♀ and I♀♀. In *L. spec. A*, first results indicate a similar shift towards higher worker ratios in EE × E — colonies of this non parasitic species. How might the caste-determination be affected by the genotypes of brood and queen? As dissections of ♀♀ from different colony types have shown, workers produced by a gynomorphic mother are less likely to have more than two ovarioles than workers reared by an intermorph. In some cases, either the allele E in the larva or the inhibiting influence of the intermorphic queen might not only prevent the development of wings, thoracic sutures, and ocelli, but of a receptacle and the ovaries, too. A larva, epigenetically predetermined to develop to a young intermorph, thus might become a worker with six or less ovarioles. ♀♀ of *H. sublaevis* regularly have 5, 6, or 7 ovarioles, and the intermorphs have 7 or 8 (BUSCHINGER and WINTER 1978). In the closely related *H. canadensis*, a species that has only gynomorphic queens, workers have 2, 3, or 4 ovarioles (BUSCHINGER and ALLOWAY, 1978).

Our observations suggest that reproductive organs develop during an earlier stage of ontogeny than external structures. If a female of *L. spec. A* has wings, she usually has the thoracic structure of a gynomorph. If she has at least slightly impressed promesonotal sutures, she regularly has ocelli, too. If there are ocelli, in all cases there is also a receptacle. Similar results have been found in *Myrmica* (BRIAN, 1955), *Leptothorax nylanderi* (PLATEAUX, 1970), and *Harpagoxenus sublaevis* (WINTER and BUSCHINGER 1986).

If our single-locus-hypothesis, or any other hypothesis with several loci, is applied to calculate allele frequencies in the Tadoussac population, in some mating types, especially ee × e, observed and expected frequencies differ significantly (table II). There are too many gynomorphic females who produce gynomorphic offspring, compared to those who rear intermorphs. With respect to queen polymorphism the population apparently is not in a Hardy-Weinberg equilibrium. Electrophoretical studies on esterase variability do not give clear evidence for inbreeding that would explain this asymmetry.

In two species of *Pogonomyrmex*, DAVIDSON (1982) observed mating preferences: large females mated disproportionately with large males. In *Leptothorax spec. A* G♀♀ and e-♂♂ are significantly larger than I♀♀ and E-♂♂. In the laboratory, males indiscriminantly seize females and attempt to

mate, but females quite often actively resist copulation by attacking the male. Size preferences in mating, similar to the relations in *Pogonomyrmex*, might lead to higher frequencies of homozygous mating types.

Gynomorphs and intermorphs of *Leptothorax spec. A* have been collected in about twenty different populations throughout Quebec and New England. The frequencies of gynomorphs seem to be correlated with characters of the biotope. In open, homogeneous forests, as in St. Siméon, in Grands Jardins, or on Mt. Monadnock, gynomorphs by far outnumber intermorphic females. In patchily distributed suitable areas, as in Matagami or Tadoussac, more than 80 percent of queens are intermorphic. Studies on colony structure in *Leptothorax* (HEINZE and BUSCHINGER, in press.) suggest, that some young females of *L. spec. A*, especially I♀♀, return into their mothers' colonies after mating. There they stay without becoming fertile (functional monogyny); new colonies perhaps are founded by budding in spring. Evidence has been found for solitary colony foundation especially by G♀♀. Intermorphic females show a stationary sexual calling behavior and most probably start sexual display in the immediate vicinity of the nest. Gynomorphs, on the other hand, fly for several meters before starting sexual calling. The habitats with high frequencies of intermorphic females have a size of only several 10 to 100 square meters, they are wind exposed, and they are surrounded by areas unsuitable for colony foundation: the sea, as on the rocky outcrops in Tadoussac and Grandes Bergeronnes, or dense thickets, forests or spruce-bogs, like Matagami. A gynomorph might frequently drift off during sexual flight behavior, and thus have a lower chance for successful colony foundation in patchy biotopes. The density of available nesting sites is quite high on the outcrops in Tadoussac or Matagami. Rotten branches, partly covered by lichens and shadowed by American laurel and other plants of the heather family, provide preformed cavities with the proper amount of insolation for *Leptothorax* ants to nest in. In Tadoussac, we collected five and more colonies from one square meter. Colony foundation by budding might be highly successful here.

On the other hand, in the light coniferous forests in Grands Jardins or St. Siméon, comparable nesting sites are quite rare and colonies of *L. spec. A* are far less abundant. Budding perhaps becomes more difficult in these habitats, and a winged gynomorph might have a better chance to find a suitable place for colony foundation than a wingless intermorph.

Gynomorphic females also play an important role in the colonization of new habitats that are inaccessible to intermorphs. In one rocky area in La Baie, apparently the ideal biotope for intermorphic females, all six colonies contained a gynomorphic queen (unpubl. res.).

Recent observations in the field indicate, that queen polymorphism is not as rare in the higher ants as was previously thought. In the genus

Monomorium, both intermorphic and gynomorphic females have been described in afrotropical *M. rufulum* (BOLTON, 1986) and *M. albopilosum* (BOLTON 1987), in *M. trageri* from Florida (DU BOIS 1986), and in the Australian species *M. rubriceps* (TULLOCH, 1930). Unpublished finds by S. COVER (pers. comm.) in North America and BUSCHINGER in Australia indicate that queen polymorphism in *Monomorium* occurs in other areas, too. Intermorphic females have been collected, e.g., in the neotropical *Aphaenogaster phalangium* (S. COVER, pers. comm.), in several insular ants (WILSON, 1971), and in the genus *Megalomyrmex* (BRANDAÓ, 1987). Both non-parasitic *Leptothorax* with queen polymorphism, *L. spec. A* and *L. sphagnicolus* (FRANCOEUR 1986), have been found in Quebec. A third taxon with intermorphic and gynomorphic females was observed in the Rocky Mountains (S. COVER, pers. comm.). It is somewhat surprising that intermorphic queens apparently are absent from the well studied leptothoracine fauna of North and Central Europe. In Northern Scandinavia and Scotland, however, in habitats similar to those of *L. spec. A* and *L. sphagnicolus* in Northeast America, *L. acervorum* is the only species of *Leptothorax* s. str. (COLLINGWOOD, 1969), a species in which mating during swarming flights has been observed (BUSCHINGER and WINTER, 1978). Intermorphic females there probably have no chance to mate.

Leptothorax spec. A, which is a common ant in vast areas of Northeast America and which can easily be bred in the laboratory, is evidently an ideal animal for the study of sociobiology, population genetics and ecological adaptation in ants.

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