Experimental Evidence for Genetically Mediated Queen Polymorphism in the Ant species Myrmecina graminicola (Hymenoptera: Formicidae)

ALFRED BUSCHINGER*

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Controlled mating of gynomorphic (***gyn/gyn***) and intermorphic (***gyn/int*** or ***int/int***) females of *Myrmecina graminicola* (Latreille 1802) with *gyn*- or *int*-males, respectively, and rearing of their sexual offspring revealed that the genotypes of the parents are decisive of the phenotypes of female progeny. Female sexuals, both gynomorphs and intermorphs, exhibit a kind of sexual calling behavior, the gynomorphs flying only little. Males are attracted by poison gland secretion of the females. Colony foundation success of mated queens was enhanced by adding a few workers from the parental colony, and worker pupae and larvae from other colonies. The first sexual offspring was reared in the colonies in the 2nd to 6th artificial summer season. In up to four "summers" always the same phenotype(s) of young queens were produced in a given colony. Gynomorphs (*gyn/gyn*) mated with a son of a gynomorph (*gyn*-♂) always produced gynomorphs (workers and a few males in addition). Intermorphs mated with a *gyn*-♂ either produced both gynomorphs and intermorphs (*gyn/gyn* and *gyn/int*, if the queen was a *gyn/int* heterozygote), or only intermorphs. Since *gyn/int*-♀♀ and *int/int*-♀♀ are morphologically indistinguishable, as well as *gyn*-♂♂ and *int*-♂♂, the putative genotype of intermorphs and *int*-♂♂ had to be identified according to the phenotypes of their mothers and later produced sisters in the laboratory-reared parental colonies.

The effects of the hypothesised alleles *gyn* and *int* are similar to *e* and *E* in *Harpagoxenus sublaevis* and *Leptothorax* sp-A in that *int* is dominant in preventing the development of gynomorphs from *gyn/int*-larvae. This is the third example of a genetically mediated queen polymorphism in an ant species confirmed by crossbreeding experiments, and the first instance in a tribe (Myrmecinini) outside the Formicoxenini. The principle may be involved in other instances of queen polymorphism among ants.

Key words: *Myrmecina graminicola* (Latreille 1802) – gynomorph – intermorphic queens – crossbreeding – controlled ant mating – sexual calling

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* In Memoriam Herrn Professor Dr Werner Peters, dem ich persönlich sehr viel verdanke. Über die Arbeit am „Lehrbuch der Entomologie" kamen wir uns näher. Die anregende und humorvolle Korrespondenz empfand ich stets als Gewinn.

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BUSCHINGER A [Inst Zool, Univ, D-64287 Darmstadt]: **Experimenteller Nachweis für genetisch bedingten Königinnen-Polymorphismus bei der Ameisen-Art Myrmecina graminicola (Hymenoptera: Formicidae)**. – Entomol Gener **27**(3/4): 185–200; Stuttgart 2005-02. – – – [Abhandlung]

Kontrollierte Verpaarung von gynomorphen (***gyn/gyn***) und intermorphen (***gyn/int*** oder ***int/int***) Weibchen von *Myrmecina graminicola* (Latreille 1802) mit ***gyn***- bzw ***int***-Männchen, sowie die Aufzucht von deren Geschlechtstier-Nachwuchs zeigte, daß die elterlichen Genotypen über den Phänotyp der weiblichen Nachkommen entscheiden. Weibliche Geschlechtstiere, sowohl Gynomorphe als auch Intermorphe, zeigen eine Art Locksterzel-Verhalten, wobei die Gynomorphen nur wenig flugaktiv sind. Männchen werden durch ein Sekret der weiblichen Giftdrüse angelockt. Der Erfolg der Koloniegründung begatteter Jungköniginnen wurde durch Zugabe einiger Arbeiterinnen aus der elterlichen Kolonie gefördert, sowie durch einige Arbeiterinnen-Puppen und -Larven aus fremden Völkern. Erste Geschlechtstier-Nachkommen wurden in den Kolonien in der zweiten bis sechsten künstlichen Sommerperiode aufgezogen. In bis zu vier ‚Sommern' wurde(n) in einer Kolonie jeweils der (die) gleiche(n) Phänotyp(en) produziert. Gynomorphe (*gyn/gyn*) erzeugten nach Verpaarung mit dem Sohn einer Gynomorphen (*gyn*-♂) immer Gynomorphe (neben Arbeiterinnen und einigen Männchen). Intermorphe produzierten nach Verpaarung mit einem *gyn*-♂ entweder nebeneinander Gynomorphe und Intermorphe (*gyn/gyn* und *gyn/int*, wenn die Königin eine *gyn/int* Heterozygote war), oder ausschließlich Intermorphe. Da *gyn/int*-♀♀ und *int/int*-♀♀ morphologisch nicht zu unterscheiden sind, ebenso wie *gyn*-♂♂ und *int*-♂♂, konnte der vermutliche Genotyp der Intermorphen und der *int*-♂♂ nur aus den Phänotypen ihrer Mütter ermittelt werden sowie aus später in den elterlichen Kolonien aufgezogenen Schwestern.

Die Auswirkungen der hypothetischen Allele *gyn* und *int* sind ähnlich denen von *e* und *E* bei *Harpagoxenus sublaevis* (Nylander 1852) und *Leptothorax* sp-A. Wie E in diesen Fällen, verhindert *int* dominant die Entstehung von Gynomorphen aus heterozygoten *gyn/int*-Larven. Dies ist das dritte bewiesene Beispiel für einen genetisch bedingten Königinnen-Polymorphismus bei Ameisen, und es ist das erste Beispiel aus einer Tribus (Myrmecinini) außerhalb der Formicoxenini. Das Prinzip könnte in weiteren Fällen von Königinnen-Polymorphismus bei Ameisen ebenfalls eine Rolle spielen.

Schlüsselbegriffe: *Myrmecina graminicola* (Latreille 1802) – Gynomorphe – intermorphe Königinnen – Kreuzungen – kontrollierte Verpaarung von Ameisen – Locksterzeln

1 Introduction

In numerous ant species, the reproductive females were found to deviate from the common phenotype of an alate or dealate fully developed 'queen', and are replaced by intermediate 'intercastes', by workerlike 'ergatoid queens', or by 'gamergates' [HEINZE 1998; PEETERS & ITO 2001].

Queen polymorphism, the coexistence of different phenotypes of reproductive females in one and the same species, however, is a rare phenomenon. And the genetic or environmental factors involved have been studied in only a few cases [BUSCHINGER & HEINZE 1992; HEINZE 1998]. Another example of queen polymorphism has been recently described in the European Myrmicinae ant species, *Myrmecina graminicola* (Latreille 1802) [BUSCHINGER & SCHREIBER 2002]. Queen polymorphism, though ultimately shaped by selection, may have two origins. It may be initiated by environmental influences directly acting on the female larvae, or a genetic factor may predispose a larva to become an alate female (gynomorph) or an intermorph, respectively, ordinary caste differentiation factors being favorable.

The paper of BUSCHINGER & SCHREIBER [2002] strongly supported the genetic hypothesis: Rearing of field collected colonies with dealate or intermorphic queens, respectively, through up to seven years, and characterization of their progeny suggested that this queen polymorphism was genetically mediated. Under variable laboratory conditions, a given colony always produced either gynomorphic females only, or intermorphic females only, or simultaneously both phenotypes of female reproductive offspring. Moreover, colonies with a gynomorphic queen produced exclusively gynomorphs, or exclusively intermorphs, whereas among the colonies with an intermorphic queen, some reared only intermorphs, others both intermorphs and gynomorphs, and none produced only gynomorphs. Similar results formerly had been obtained with *Harpagoxenus sublaevis* (Nylander 1852) and *Leptothorax* sp-A [BUSCHINGER 1978; WINTER & BUSCHINGER 1986; HEINZE & BUSCHINGER 1989]. The putative genetic origin of this queen polymorphism in *M graminicola* is studied in more depth with crossbreeding experiments here.

Here as in BUSCHINGER & SCHREIBER [2002], the functional caste definition is applied. 'Queen' means a functional, mated and reproductive female, irrespective of its phenotype. The different phenotypes of female reproductives are designated 'gynomorph' (= G, alate or dealate female) and 'intermorph' (= I, morphologically between gynomorph and ordinary worker, never winged). For discussions of caste terminology see BUSCHINGER & WINTER [1976], BUSCHINGER & CROZIER [1987], BUSCHINGER & HEINZE [1992], BUSCHINGER & SCHREIBER [2002], HEINZE [1998], KELLER [1993], WINTER & BUSCHINGER [1986].

In the two examples studied earlier, *H sublaevis* and *L* sp-A, a simple Mendelian two-allele mechanism had been demonstrated, in which the allele for the formation of intermorphs is dominant. This gene, however, does not affect the (haploid) males who all are alate and morphologically identical, independent of whether they carry one or the other allele. Therefore it is quite difficult to interprete the results, compared to ordinary instances where both genders can be classified as heterozygous, or homozygous for either allele, based on their phenotypes. Also, in the cases of *H sublaevis*, *L* sp-A, and now *M graminicola*, no characters were found to discriminate between homozygous and heterozygous intermorphs.

Hence, crossmating experiments were made with sexuals of *M graminicola*, with the aim to examine the hypothesis of a genetical basis of queen polymorphism in this species, too.

BUSCHINGER & SCHREIBER [2002] had termed the hypothetical alleles 'gyn' and 'int' instead of 'e' and 'E' in *H sublaevis* and *L* sp-A, where 'E' was derived from its effect in dominantly determining the formation of 'ergatoid', workerlike females. The term 'ergatoid female' is better replaced by 'intermorph' because even the most workerlike 'ergatoid' specimens exhibit some rudimentary characters of the gynomorph, e.g traces of ocelli. Anatomically, the intermorphs in all instances are characterized by the presence of a spermatheca, and of ovaries of the same size as in gynomorphs, whereas true workers ("ergatomorphs") usually have only two ovarioles and lack a spermatheca. In addition, it is undetermined whether or not e/E in the examples of Formicoxenini, and gyn/int in the tribe Myrmecinini, refer to homologous genes.

The following scheme summarizes the hypothesis for *Myrmecina graminicola*, and the expected results of crossmatings:

♀ phenotype (*presumptive genotype*)	Mated with ♂ of (*presumptive genotype*)	Expected phenotype(s) and (*presumptive genotype(s)*) of ♀ progeny
Gynomorph (*gyn/gyn*)	(*gyn*)	Gynomorphs (*gyn/gyn*)
Gynomorph (*gyn/gyn*)	(*int*)	Intermorphs (*gyn/int*)
Intermorph (*gyn/int*)	(*gyn*)	Gynomorphs (*gyn/gyn*) and Intermorphs (*gyn/int*)
Intermorph (*gyn/int*)	(*int*)	Intermorphs (*gyn/int*) and (*int/int*)
Intermorph (*int/int*)	(*gyn*)	Intermorphs (*gyn/int*)
Intermorph (*int/int*)	(*int*)	Intermorphs (*int/int*)

For the sake of convenience, the terms for the hypothetical alleles *gyn* and *int* involved in the polymorphism of female reproductives shall be used throughout this paper, because a correct descriptive denomination of specimens would be much more awkward to write and understand (e.g, 'son of a gynomorph' instead of 'gyn-♂', or 'son of an intermorphic queen having produced exclusively intermorphic daughters' instead of 'int-♂'). This must not be misunderstood as a circular reasoning: here it is attempted to experimentally find evidence pro or contra this hypothesis.

2 Material and methods

2.1 Origin of the experimental colonies

The original colonies (identification numbers 15xxx) used in the experiments had been collected in southern Hesse and northern Bavaria, Germany [Buschinger & Schreiber 2002]. Nests and rearing conditions are detailed in this paper. It is possible, with the data in Buschinger & Schreiber [2002], to trace the genealogy of practically all specimens used for the crossbreeding experiments in the present paper (a few exceptions are mentioned in the footnotes to tables). The tables in Buschinger & Schreiber [2002] comprise the identification numbers of field-collected colonies in the collection of the first author, and they indicate which type of progeny had been reared from these colonies. The same numbers appear under 'colonies of origin' in the tables of the present paper.

2.2 Mating and rearing conditions

The sexual behavior of *M graminicola* has been described by Buschinger [2003]. Male and/or female sexuals left the nests in about the 13th week in laboratory summer conditions (temperature rhythm 18/23 °C). Formicaries with such colonies were placed singly into transparent plastic 'flight cages' (15 cm x 20 cm x 30 cm w x d x h) with a vertical sliding door in the front. 2–3 cardboard strips (ca 1 cm wide, 30 cm long and bent on top such that a platform of 1 x 3–4 cm was formed) connected the nest chambers with the rear wall of the cage which was exposed to daylight or a strong incandescent light (200 W). At temperatures of 23–25 °C the female sexuals slowly climbed up the cardboard strips where they exhibited a kind of sexual calling behavior, alate females sometimes flying for a short distance. Males were kept separate until a number of females seemed sexually active, then were released in the flight cage. When a couple was mating, it was taken out with a forceps and separated in a vial until mating was finished. Single males were able to mate with up to three females, but usually were used only once.

Mated females one or two days later were put singly into formicaries, together with 3–5 workers from their parental nests and 5–6 worker pupae and as many larvae from large stock colonies. After the first workers had hatched from the pupae, additional 15–50 foreign worker pupae were added (number depending upon availability). In the year of foundation the young queens laid some eggs that developed into small larvae until hibernation.

After 5–6 months of hibernation (at 10 °C) a "spring" period of 3 weeks (10/20 °C) followed, then the artificial 'summer' of 13 weeks (at 18/23 °C). In this spring and summer conditions the foreign larvae developed into workers (and surprisingly often into sexuals), and also the first few (ca 5–10) 'own' workers hatched ("first year"). In the following years workers were regularly reared, and from the 2nd, 3rd or 4th year on (sometimes later) also female sexual progeny of the young queens appeared. A few colonies were kept alive for up to 7 artificially compressed years, and up to 4 sexual broods were obtained from individual colonies.

The colonies were checked once a week for brood development, except during hibernation. They were censused when female sexual pupae or adult sexuals were present. The new sexuals were removed and preserved in ethanol.

2.3 Relations of phenotypes and presumptive genotypes

Only gynomorphs were directly attributable to the genotype *gyn/gyn*, and sons of gynomorphs were considered *gyn*-♂♂. Intermorphic daughters of a gynomorph, according to the underlying hypothesis, should represent the genotype *gyn/int*, and when mated with a *gyn*-♂ they should produce both gynomorphs (*gyn/gyn*) and heterozygous intermorphs (*gyn/int*).

Field colonies with an intermorphic queen, producing exclusively intermorphs, were supposed to produce *int*-♂♂ (though a *gyn/int*-intermorph mated with an *int*-♂ also would produce exclusively intermorphs, and both *gyn*- and *int*-♂♂). Field colonies with intermorphic queens and producing both gynomorphs and intermorphs should comprise a *gyn/int*-queen mated with a *gyn*-♂, and should rear both *gyn*- and *int*-♂♂.

Since there was no means to reliably identify the genotype of the sons of intermorphs, a number of experiments were run with gynomorphs mated with male offspring of intermorphic queens. Assuming that both genotypes of males would be fairly equally frequent, some of the gynomorphs should have *gyn/int* intermorphic progeny, others should yield *gyn/gyn* gynomorphs.

Because of the very slow growth of incipient *M graminicola* colonies (5 artificially compressed years needing at least 45 months or > 3½ natural years) it was not possible first to determine the genotype of an *int/int*-queen by crossmating her intermorphic progeny with *gyn*-♂♂, and then, 2–3 years later when these daughter colonies produced only intermorphs, use the *int*-♂♂ progeny of the original queen for further crossmating experiments.

3 Results

3.1 Crossbreeding experiments suggesting genotypic morph determination

Tab 1–5 reveal the results of the various breeding experiments that all support the hypothesis of a genetically mediated queen polymorphism. A few additional comments to the tables shall be given here.

– Ten gynomorphic (*gyn/gyn*-) females mated with sons of gynomorphs (*gyn*-males) all produced exclusively gynomorphs (workers and sometimes males in addition, as in all experimental colonies) (**Tab 1**).

Tab 1: Production of female sexuals in colonies of *Myrmecina graminicola* (Latreille 1802) with a *gyn/gyn* queen x *gyn*-male [Hymenoptera: Formicidae]. **G** gynomorphic female; ***gyn*-♂** son of a gynomorph.

Colony identification # (year/ col. #)	Colonies of origin: G ♀ x gyn-♂	Type and n of ♀♀ produced (n ♀♀ in 1, 2, 3 or 4 broods)	First production of ♀♀ in nth year	n broods with ♀-production / n broods total	n ♀♀ per successful brood
99/6	15749 x 15741	GG 19 (3 + 16)	4th	2/5	19:2 = 9.5
99/7	15741 x 15741	GG 4 (4)	3rd	1/5	4:1 = 4
99/8	15749 x 15748	GG 58 (3+17+19+19)	2nd	4/5	58:4 = 14.5
99/9	15749 x 15748	GG 58 (3+24+31)	3rd	3/5	58:3 = 19.3
99/11	15749 x 15752	GG 60 (25+35)	4th	2/5	60:2 = 30
00/2	15770 x 15741	GG 81 (4+26+51)	2nd	3/4	81:3 = 27
00/3 [a])	15770 x 15741	GG 42 (8+5+29)	2nd	3/4	42:3 = 14
00/4	15770 x 15741	GG 89 (2+19+68)	2nd	3/4	89:3 = 29.6
00/5 [a])	15770 x 15741	GG 47 (5+42)	3rd	2/4	47:2 = 23.5
00/8	15770 x 15741	GG 56 (10+46)	3rd	2/4	56:2 = 29
n = 10 colonies			**Ø 2.8th**	**25/45 = 0.55**	514 G-♀♀; 51.4 / colony; 11.4 per brood (all broods); **20.56 G-♀♀ per successful brood**

[a]) 00/3 and 00/5: same male.

– Fourteen out of twenty-two gynomorphs mated with sons of intermorphic queens produced gynomorphs (**Tab 2**), eight produced intermorphs (**Tab 3**). The genotypes of the males (*gyn* or *int*) were unknown. Since several of the gynomorphs had mated with the same males, the number of males that had sired intermorphs is reduced to seven, that of males having sired gynomorphs, to ten. No gynomorph produced both female phenotypes simultaneously.

– Six heterozygous intermorphs (*gyn/int*, five of them daughters of gynomorphic queens) inseminated by *gyn*-males all produced both gynomorphs and intermorphs simultaneously (**Tab 4**).

– Another colony, 98/9, producing both G- and I-females, had an I-queen (daughter of an I-queen producing both G- and I-females, field col. # 15547), inseminated by a male of uncertain genotype. The remaining five colonies produced exclusively intermorphs: Col 98/17, 98/18 and 98/20, intermorphic daughters of an I-queen (# 15602, having produced only intermorphs), col. 99/1 with an intermorphic daughter of an I-queen (# 15601, having produced G- and I-females), mated with a son of an I-queen (# 15602, having produced only intermorphs), and col 00/21 with an intermorphic daughter of field colony # 15698 (with I-queen producing only intermorphs), mated with a *gyn*-male (**Tab 5**).

Apart from the colonies in **Tab 2** and **3** where single males sometimes had inseminated two or three females of identical phenotype, with always the same result, another type of crossmatings is of considerable interest: Here, one and the same male had sucessively inseminated a gynomorphic and an intermorphic female. This was possible only twice, because other suitable females and males were not simultaneously available. However, in 98/16 (**Tab 2**) the male produced gynomorphs with a gynomorph, in 98/17 (**Tab 5**) this same male had sired intermorphs with an intermorphic queen; in 98/19 (**Tab 2**) a male had produced gynomorphs with a gynomorph, in 98/20 (**Tab 5**) the same male with an intermorph had intermorphic progeny.

3.2 Lack of environmental effects on morph determination

No particular experiments were run to investigate the possibility of a phenotypic determination of queen morphs. However, a couple of casual observations revealed that this possibility in *M graminicola* may be dismissed:

– As is explained in the materials and methods, all incipient colonies received a small number of larvae from foreign, large colonies. Numbers and origin of these larvae were recorded. Quite frequently these larvae not only developed into workers during the "first year" of the colony, but yielded a few female sexuals instead. The phenotypes of these sexuals in all instances corresponded to those produced in their colonies of origin.

– In a number of early experiments, field-collected colonies were dequeened, or split into a queenright and a queenless subcolony, mainly in order to increase the production of female sexuals by reduction of the inhibitory queen effect. This works well in many ant species. However, none of the queenless subcolonies ever reared a female phenotype different from that produced in the queenright subcolony (and dequeened colonies did not yield markedly higher numbers of female sexuals).

Tab 2: Production of female sexuals in colonies of *Myrmecina graminicola* (Latreille 1802) with a ***gyn/gyn* queen** x **(?)*gyn*-male** [Hymenoptera: Formicidae]. – (Males were taken from field colonies with one or several I-queens having produced both G and I-♀♀. Thus, males could be either *gyn* or *int,* but were identified as *gyn* because of their *gyn/gyn* offspring). **G** gynomorphic female, **I** intermorphic female.

Colony identification # (year/ col. #)	Colonies of origin: G-♀ x (?)♂	Type and n of ♀♀ produced (n ♀♀ in 1, 2, 3 or 4 broods)	First production of ♀♀ in nth year	n broods with ♀-production / n broods total	n ♀♀ per successful brood
98/4	15526 x 15547	GG 36 (2+1+33)	2nd	3/6	36:3 = 12
98/5 [a])	15526 x 15546	GG 10 (1+4+5)	2nd	3/4	10:3 = 3.3
98/6 [b])	15526 x 15546	GG 27 (15+3+9)	3rd	3/6	27:3 = 9
98/7 [c])	15526 x 15546	GG 26 (1+25)	3rd	2/4	26:2 = 13
98/8	15526 x 15546	GG 3 (1+2)	3rd	2/4	3:2 = 1.5
98/10 [a])	15526 x 15546	GG 39 (39)	6th	1/6	39:1 = 39
98/11 [a])	15526 x 15546	GG 60 (1+59)	4th	2/6	60:2 = 30
98/12 [c])	15526 x 15546	GG 30 (14+16)	3rd	2/4	30:2 = 15
98/14 [b])	15526 x 15546	GG 50 (15+35)	3rd	2/4	50:2 = 25
98/16 [d])	15601 x 15618	GG 42 (4+2+36)	2nd	3/4	42:3 = 14
98/19 [e])	15601 x 15618	GG 26 (7+3+16)	2nd	3/4	26:3 = 8.6
99/15	15749 x 15732	GG 34 (4+30)	2nd	2/5	34:2 = 17
00/6	15770 x 15750	GG 17 (17)	2nd	1/2	17:1 = 17
00/7	15770 x 15750	GG 16 (16)	4th	1/4	16:1 = 16
n = 14 colonies			**Ø 2.7th**	**30/63 = 0.47**	416 G-♀♀; Ø 29.7/ colony; 6.6/ brood (all broods); **13.9 G-♀♀ per successful brood**

[a]) 98/5, 98/10, 98/11: same male; [b]) 98/6 and 98/14: same male; [c]) 98/7 and 98/12: same male;

[d]) The queens of 98/16 (G-♀) and 98/17 (I-♀, tab 5) had mated with the same male. The male was a *gyn*-♀ having sired G-♀♀ with G-♀ 98/16, and I-♀♀ with I-♀ 98/17.

[e]) The queens of 98/19 (G-♀) and 98/20 (I-♀, tab 5) had mated with the same male. The male was a *gyn*-♀ having sired G-♀♀ with G-♀ 98/19, and I-♀♀ with I-♀ 98/20.

Tab 3: Production of female sexuals in colonies of *Myrmecina graminicola* (Latreille 1802) with a *gyn/gyn* queen x (?)*int*-male [Hymenoptera: Formicidae]. – (Males were taken from field colonies with one or several I-queens having produced both G and I-♀♀. Thus, males could be either *gyn* or *int* but were identified as *int* because of their *gyn/int*-offspring). **G** gynomorphic female, **I** intermorphic female.

Colony identification # (year/ col. #)	Colonies of origin: G-♀ x (?)♂[b])	Type and n of ♀♀ produced (n ♀♀ in 1, 2, 3 or 4 broods)	First production of ♀♀ in nth year	n broods with ♀-production / n broods total	n ♀♀ per successful brood
98/2 [a])	15526 x 15527	II 5 (5)	4th	1/6	5:1 = 5
98/3 [a])	15526 x 15527	II 38 (38)	6th	1/6	38:1 = 38
98/22 [b])	15668 x 15666	II 5 (1+3+1)	3rd	3/5	5:3 = 1.6
98/23 [b])	15668 x 15666	II 6 (3+1+2)	3rd	3/5	6:3 = 3
98/26	15668 x 15669	II 25 (25)	6th	1/6	25:1 = 25
00/9	15770 x 15732	II 37 (37)	4th	1/4	37:1 = 37
00/10	15770 x 15732	II 40 (40)	4th	1/4	40:1 = 40
00/11	15770 x 15732	II 42 (42)	4th	1/4	42:1 = 42
n = 8 colonies			**Ø 4.25th**	**12/40 = 0.3**	198 I-♀♀; Ø 24.75/ colony; 4.95/ brood (all broods); **16.5 I-♀♀ per successful brood**

[a]) 98/2 and 98/3: same male

[b]) All males in Tab 3 were from colonies with I-queen(s) producing both G- and I-offspring, except for colony # 15666 with **I**-queens that in 6 laboratory broods only once had produced 2 intermorphs.

Tab 4: Production of female sexuals in colonies of *Myrmecina graminicola* (Latreille 1802) with an intermorphic queen *(gyn/int)* x *gyn*-male [Hymenoptera: Formicidae]. – (*gyn*-♂♂ were taken from field colonies with a gynomorphic queen). **G** gynomorphic female, **I** intermorphic female.

Colony identification # (year/ col. #)	Colonies of origin: G-♀ x gyn-♂	n of ♀♀ Type and and produced (n G-, n I-♀♀ in 1, 2 or 3 broods)	First production of ♀♀ in nth year	n broods with ♀-production / n broods total	n ♀♀ per successful brood G-♀♀ I-♀♀
99/16 [c])	15746 x 15741	52 G, 38 I (2 G, 2 I+2 G, 2 I+24 G, 21 I+24 G, 13 I)	2nd	4/5	52:4=13 38:4=9.5
99/19	15748 x 15741	22 G, 24 I (1G,1I+20G,23I+1G)	2nd	3/5	22:3=7.3 24:3=8
99/20	15748 x 15741	36 G, 28 I (4G, 1I+28G, 27I+4G	2nd	3/5	36:3=12 28:3=9.3
00/17 [a]) [b])	15635 x 98/25	8 G, 9 I (7G, 9I+1G)	2nd	2/3	8:2=4 9:2=4.5
00/18 [b])	15635 x 15636	17 G, 21 I (5G,9I+12G,12 I)	2nd	2/3	17:2=8.5 21:2=10.5
00/20 [b])	15635 x 15636	3 G, 9 I (2G,3I+1G,6I)	2nd	2/3	3:2=1.5 9:2=4.5
n = 6 colonies			Ø 2nd	16/24 = 0.66	**138 G** = 8.62 G-♀♀ per successful brood **+ 129 I** = 8.06 I-♀♀ per successful brood
					267 ♀♀ total (G + I); Ø 44.5 per colony; 11.12/ brood (all broods); **Ø 16.69 per successful brood.**

[a]) The 'queen' of 98/25 was a virgin **G-**♀.
[b]) Field colony #15635 (not included in Buschinger & Schreiber 2002] had a **G-queen** producing **G**- and **I-**♀♀. The latter were used in these experiments.
[c]) #15746 had an I-queen producing only **I-**♀♀. This queen probably was *int/int*, mated with a ***gyn*-**♂. The **I-queen** of col. 99/16 hence was probably ***gyn/int***.

Tab 5: Production of female sexuals in colonies of *Myrmecina graminicola* (Latreille 1802) with an intermorphic queen (*gyn/int* **or** *int/int*) x *gyn*- **or** *int*-male [Hymenoptera: Formicidae]. – (I-females were taken from colonies with I-queens having produced both G- and I-females, #15547 and #15601, and from colonies with I-queens having produced only I-females, #15602 and #15698; males were taken from field colonies with one or several I-queens having produced only I-♀♀. Males could have been *gyn* or *int*). One colony produced both G- and I-♀♀, five only I-♀♀, none only G-♀♀. (**G** gynomorphic female, **I** intermorphic female).

Colony identification # (year/ col. #)	Colonies of origin: I-♀ x (?)♂	Types and n of ♀♀ produced (n G-, n I-♀♀ in 1, 2 or 3 broods)	First production of ♀♀ in nth year	n broods with ♀-production / n broods total	n ♀♀ per successful brood
98/9 [a])	15547 x 15546	11G, 10 I (2 G, 1 I + 9 G, 9 I)	4th	2/5	21:2=10.5
98/17 [b])	15602 x 15618	7 I (4+3)	3rd	2/5	7:2=3.5
98/18	15602 x 15618	20 I (2+7+11)	2nd	3/6	20:3=6.6
98/20 [c])	15602 x 15618	6 I (3+3)	3rd	2/6	6:2=3
99/1	15601 x 15602	29 I (29)	5th	1/5	29:1=29
00/21	15698 x 15636 (gyn-♂)	32 I (14 I+18 I)	2nd	2/3	32:2=16
n = 6 colonies			Ø 3.16th	12/30 = 0.4	115 ♀♀;19.2 / colony; 3.8/ brood (all broods); **9.6 ♀♀ per successful brood**

[a]) #15547 with two I-queens [not included in Buschinger & Schreiber 2002] had produced G- and I-♀♀. The I-queen of colony 98/9 thus was probably *gyn/int*.
[b]) The queens of 98/17 (I-♀) and 98/16 (G-♀, Tab 2) had mated with the same male.
[c]) The queens of 98/20 (I-♀) and of 98/19 (G-♀, Tab 2) had mated with the same male.

3.3 Failed experiments

Quite a number of experiments failed in that the young queens were not properly inseminated (which was checked by dissection), died from unknown reasons, or did not produce female sexuals during up to seven artificial years. In total thus, only 44 out of 70 incipient colonies were 'successful' in that they produced female sexuals in sufficient numbers. In nine of the 26 colonies that 'failed' the 'queens' were not inseminated (six G-♀♀, three I-♀♀); in further ten colonies the queens died before they could produce sexual offspring (five G-♀♀, five I-♀♀); and in seven colonies no or only few female sexuals were reared, though these colonies were kept for up to seven "years", and all had produced worker offspring (three G-♀, four I-♀♀).

3.4 Quantitative aspects

Tab 1–5 comprise a number of semiquantitative results. As was mentioned in materials and methods, a sound quantitative evaluation is impossible due to changing conditions during the five years of breeding, with improvements achieved mainly in the colonies founded in 1999 and 2000. Several data, however, at least tentatively suggest that differences between the various genotypes are small.

i) The first production of female sexuals occurred in the 2nd to 6th year after colony foundation. In the tables a mean value is indicated which is close to 3rd, for G-producing gynomorphs (*gyn/gyn* x *gyn*, **Tab 1 & 2**). This value is ca 4th (**Tab 3**) for I-producing gynomorphs (*gyn/gyn* x *int*), and 2nd (**Tab 4**) for intermorphs producing both G- and I-offspring (*gyn/int* x *gyn*). Colonies with *gyn/gyn*-larvae seem to rear female sexuals earlier than those with *gyn/int*-larvae (**Tab 3**). However, the brood in **Tab 4** comprises both *gyn/gyn*- and *gyn/int*-larvae, and in all colonies sexuals of both genotypes appeared already in the 2nd brood. Unfortunately no data exist from clearly identified *int/int* x *int*-colonies, hence the development of *int/int*-larvae.

ii) A second set of data refers to the fraction of "successful" broods (i.e. those where female sexuals were reared), compared to "all broods" (including the 1st which never yielded sexual offspring of the queens). This fraction varies from 0.55 and 0.47 in *gyn/gyn* x *gyn*-colonies (**Tab 1 & 2**) to 0.3 in *gyn/gyn* x *int*-colonies (**Tab 3**) and 0.66 in *gyn/int* x *gyn*-colonies (**Tab 4**). Because of low numbers of colonies, however, the differences may be accidental.

iii) Finally, the numbers of female reproductives produced per 'successful' brood were calculated in **Tab 1–5**. The results are compiled in **Tab 6**. Since individual colonies were reared for 2–6 years, the comparison of total production per colony would be biased. The production of G- and I-♀♀ is not much different. Particularly the six colonies rearing both G- and I-♀♀ (**Tab 4**) had both phenotypes in practically equal numbers (138 G-♀♀ and 129 I-♀♀; $\chi^2 = 6.448$; P = 0.264; NS).

The marked difference between the two groups rearing G-♀♀ (**Tab 1 & 2**, 20.56 vs. 13.9 ♀♀) most probably is due to the fact that **Tab 1** comprises only colonies founded in 1999 and 2000 (hence with 'better' rearing conditions), whereas most of the colonies in **Tab 2** had been founded in 1998 (with considerably less worker pupae added to the incipient colonies).

The number of workers produced in the genetically different colonies was also recorded. However, they were so highly variable that it was impossible to find any correlation between worker number and colony genotype.

Tab 6: Comparison of productivity (n G- and/or I-♀♀ per successful brood) in colonies with different genotypes of *Myrmecina graminicola* (Latreille 1802) [Hymenoptera: Formicidae].

Tab #	(n colonies)	Genotype of ♀♀	Genotype of ♂♂	Ø Production G- and/or I-♀♀
1	(10)	*gyn/gyn*	gyn	20.56 G
2	(14)	*gyn/gyn*	*gyn*	13.9 G
3	(8)	*gyn/gyn*	*int*	16.5 I
4	(6)	*gyn/int*	*gyn*	16.7 G + I (8.62 G + 8.06 I)
5	(6)	*gyn/int* or *int/int*	*gyn* or *int*	9.6 G + I

4 Discussion

4.1 The hypothesis of a genetically mediated queen polymorphism in M graminicola

Breeding a species like *M graminicola*, with its slow succession of generations, and long annual cycles, is a laborious venture. It was not possible, over the years, to keep rearing conditions ideally constant. Quantity and quality of food, temperature conditions etc. were successively adapted with the aim of increasing the production of female sexual offspring. Particularly the numbers of worker pupae from stock colonies given to incipient colonies were dependent on availability, but were also higher in the later experiments. Higher numbers of added worker pupae resulted in an earlier production of female sexuals and increased their numbers. Hence, quantitative comparisons of colonies with differing genotypes can only be made with precaution, statistical comparisons of results from earlier and later founded colonies would be misleading.

In spite of the problems of quantitative evaluation, the qualitative results, production of merely gynomorphs, merely intermorphs, or both in a colony, remained fairly constant, and in **all** instances fit perfectly to the hypothesis of a genetically mediated polymorphism of the presumptive queens (see section 3.1). The results all are best explained by the hypothesis of a genetic mechanism underlying queen polymorphism (cf scheme in the Introduction), as had been suggested by BUSCHINGER & SCHREIBER [2002].

4.2 Intermorphic queens and queen polymorphism in other Myrmecina-species?

Little is known on queen phenotypes in other *Myrmecina* species. The Japanese congener, *Myrmecina nipponica*, a very close relative of *M graminicola*, also exhibits a queen polymorphism. However, according to MURAKAMI et al [2000], this species has two types of colonies, *"a queen colony type, in which the reproductive females are queens* [i.e gynomorphic queens as defined in this paper] *... and an intermorphic female colony type, in which reproductive females belong to a wingless intermediate morphology between queen and worker* [i.e intermorphic queens as defined in this paper] *...". "The gene flow between the two types of colonies is mediated by males, which can inseminate intermorphic females as well as queens".*

MURAKAMI et al [2002] confirm this strict separation of colonies with gynomorphic and intermorphic queens, respectively, though *"intermorphic queens in M. nipponica occasionally coexist with alate/dealate queens in a single colony"* as had been reported by OHKAWARA et al [1993] [these authors used the term 'intercastes' instead of 'intermorphic queens']. Furthermore, *"...the emergence of intermorphic queens in alate/dealate queen right colonies is a rare event in this ant species, and the origin of intermorphic queens is unknown"*. In detail, *"... such coexistence* [of intermorphic queens with alate/dealate queens] *has been observed in only 5 % of all alate/dealate queenright colonies"* [MURAKAMI et al 2002].

Unfortunately it remains unclear whether 'coexistence' here means the production of intermorphs by reproductive gynomorphic or intermorphic queens, or the coexistence of virgin specimens of the two phenotypes, or something else. It is also not possible here to figure out whether and why *M nipponica* and *M graminicola* differ that much in the proportions of colonies with the two phenotypes of female reproductives, and whether or not queen polymorphism in *M nipponica* also is genetically mediated. Queen phenotype apparently is variable among the species of the genus. ITO [1996] reported on a *Myrmecina* sp from Indonesia (Java), where only *"ergatoid queens"* [= intermorphic queens] were found.

4.3 Arguments against an epigenetic polymorphism

Caste differentiation, the decision on the development of female larvae into workers or female sexuals, is achieved in social Hymenoptera by nutritional effects [HÖLLDOBLER & WILSON 1990], though recent studies occasionally suggest genetic factors to be involved e.g in worker polymorphism [HUGHES et al 2003].

In the two instances of queen polymorphism that as yet have been studied with crossbreeding experiments, *Harpagoxenus sublaevis* and *Leptothorax* sp-A, genetical mechanisms have been proven [WINTER & BUSCHINGER 1986; HEINZE & BUSCHINGER 1989]. One other instance of queen polymorphism is the Australian *Monomorium rubriceps* Mayr 1876, in which all field colonies had an intermorphic queen, and all produced exclusively intermorphic female reproductives. Only when a colony lost its queen, alate gynomorphs were reared from the remaining brood [BUSCHINGER & HEINZE 1992; here referred to as *Monomorium* sp-2]. Thus, queen polymorphism apparently can be mediated by both genetic control or by environmental factors.

For *M graminicola*, the crossbreeding results presented in this paper strongly support a genetic mechanism. A few casual observations in addition speak against an epigenetic influence on the formation of either gynomorphs or intermorphs (see section 3.2).

The experiments reported here, however, did not explain the high morphological variation within the intermorphs as described in BUSCHINGER & SCHREIBER [2002]. Intermorphs may be very similar to ergatomorphs, having only the larger compound eyes like gynomorphs, or they may be more intermediate up to a phenotype nearly looking like a wingless gynomorph.

The laboratory founded colonies produced mainly 'lower' or intermediate intermorphs, very rarely a specimen that resembled a gynomorph. In field colonies such 'higher' intermorphs are more frequent. Since both in field and laboratory colonies, however, a certain variation was found also among the intermorphic progeny of single queens, it may be suggested that nutritional or other environmental factors have an additional modifying influence on the shape of the intermorphs.

4.4 Queen polymorphism and caste differentiation

Queen polymorphism in ants is comparable to the frequent instances of genetically mediated wing dimorphism in other insects [e.g ROFF 1986], and may have similar ecological meaning with respect to dispersion capacity; for discussion see PEETERS & ITO [2001] and BUSCHINGER & SCHREIBER [2002]. In social Hymenoptera, particularly in ants with their wingless worker caste, the genetical basis of queen polymorphism apparently interferes with ordinary caste differentiation, the decision on the formation of workers and/or female reproductives as well [WINTER & BUSCHINGER 1986]. The genetic mechanisms found in *Harpagoxenus sublaevis*, *Leptothorax* sp-A, and now in *Myrmecina graminicola*, other than in non-social insects do not affect wing formation in males. This fact suggests close relations between caste differentiation (which is restricted to the female gender) and the differentiation of two queen phenotypes.

In *Harpagoxenus sublaevis,* the alleles 'e' and 'E' are resposible not only for the formation of the two female reproductive phenotypes (ee: gynomorph; eE and EE: intermorphs), but they also have a strong influence on the ratios of workers and female sexuals produced. 'E' slows down larval development, and reduces the numbers of both EE- and eE- sexuals produced in favor of a higher worker formation [WINTER & BUSCHINGER 1986]. In *Myrmecina graminicola* such an effect was not demonstrable (**Tab 6**), but this may be due to low colony numbers and to the lack of clearly identified *int/int* x *int*-colonies (corresponding to EE x E in *H sublaevis*).

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Author's Address – Anschrift des Verfassers: Professor Dr Alfred Buschinger, Institut für Zoologie der TU Darmstadt, Schnittspahnstraße 3, D-64287 Darmstadt, B R Deutschland.