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ANT CHROMOSOMES

I. — THE GENUS *FORMICA* (*)

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SUMMARY

Chromosome numbers of 14 species and mitotic chromosomes of 12 species of the genus *Formica* are presented. Six species of the subgenus *Serviformica* have 54 chromosomes in the diploid set, one species has 52 chromosomes. Seven species of the subgenera *Formica*, *Cryptoformica*, and *Raptiformica* have 52 chromosomes in the diploid set. The range of lengths of the chromosomes is (for all species) between about 0.7 and 1.5 μ . Most of the chromosomes are medio- or submediocentric. In four species, the amount of DNA per nucleus was determined by Feulgen measurements. Four different values were obtained, the highest in *Formica (Raptiformica) sanguinea*, medium values in *Formica (Formica) polyctena* and *Formica (Formica) lugubris* (these two species have similar amounts of DNA), and the lowest value in *Formica (Serviformica) fusca*. Together with morphological and ecological results, our data indicate that the subgenus *Serviformica* is well-separated from the other three subgenera and is the most primitive one. The comparison of the chromosome numbers and the DNA amounts per nucleus in the genus *Formica* and between *Formica* and *Lasius* show once more that these two characters can evolve independently.

ZUSAMMENFASSUNG

Ameisenchromosomen. I. — Die Gattung *Formica* *

Die Karyotypen von 12 Arten der Gattung *Formica* wurden untersucht. Von zwei weiteren Arten dieser Gattung wurden nur die Chromosomenzahlen bestimmt. Sechs Arten des Subgenus *Serviformica* haben diploid 54, eine Art 52 Chromosomen. Sieben

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Arten der Subgenera *Formica*, *Coptoformica* und *Raptiformica* haben ebenfalls 52 Chromosomen diploid. Die Chromosomenlängen für alle Arten liegen zwischen 0,7 und 1,5 µm. Die meisten Chromosomen aller Arten sind medio- oder submediozentrisch. Bei vier Arten wurde die relative DNS-Menge diploider Gehirnzellen bestimmt mit Hilfe von Feulgen-Absorptionsmessungen. Es ergaben sich vier verschiedene Werte, der höchste bei *Formica (Raptiformica) sanguinea*, zwei ähnlich grosse mittlere Werte bei *Formica (Formica) polyctena* und *Formica (Formica) lugubris* und der niedrigste Wert bei *Formica (Serviformica) fusca*. Zusammen mit morphologischen und ökologischen Daten weisen unsere Resultate darauf hin, dass die Untergattung *Serviformica* von den drei übrigen Untergattungen gut abgetrennt ist und am ursprünglichsten scheint. Der Vergleich von Chromosomenzahlen und DNS-Werten innerhalb der Gattung *Formica* und zwischen den Gattungen *Formica* und *Lasius* zeigt einmal mehr, dass sie getrennt evolviieren können.

INTRODUCTION

In Japan (IMAI, 1966, 1969; IMAI and YOSIDA, 1964), in America and Australia (reviewed by CROZIER, 1970; HUNG, 1969) and in Europe (HAUSCHTECK, 1961, 1962), Formicidae karyotypes have been studied. The present data concern the genus *Formica*. The investigated species of the genus *Formica* belong to four subgenera: *Serviformica*, *Formica*, *Coptoformica*, and *Raptiformica*. The morphological similarity of the species in the subgenera, especially in the subgenus *Formica*, is one of the problems in ant taxonomy. IMAI and YOSIDA (1964), IMAI (1966, 1969), and HUNG (1969) have published some *Formica* chromosome numbers and IMAI (1969) has published one karyotype. The present paper gives more karyotypical information on *Formica*. Some DNA measurements give a new perspective on evolution in the taxon.

MATERIAL AND METHOD

Karyotype analysis.

Brains of prepupae from all castes available were dissected. Brains were kept in sterile insect ringer with 0.004 % Colcemid for two to three hours. If male prepupae were present, the testes were also studied. In one case, the ovary of an inseminated female was examined. In the gonads of adult males and in uninseminated adult females we found no mitotic or meiotic figures. After incubation in Colcemid, the dissected organs were transferred to a 1 % sodium citrate solution for 20 to 40 min. Then they were fixed in 50 % acetic acid and stained with acetic orcein, squashed under a silico-nized coverslip, which was removed after being frozen on dry ice. The cells were mounted in Euparal.

Ten mitoses with the same chromosome number are enough to determine the diploid or haploid chromosome number in this material. Therefore, in all those

species in which more than ten nuclei were analysed, the number of chromosome sets studied is listed as being larger than ten. Where the documentation of the chromosome set as karyotype or metaphase plate is missing, the preparations were destroyed or unsatisfactory. The photos were taken with a Zeiss Ultraphot II, objective Neofluar Ph100.

Relative DNA amount per nucleus.

The specimens for DNA determination were collected in or in the near of Zürich. Only brains of adult workers were dissected because all cells of this organ in adults are in G₀-phase. The brains were fixed in 50 % acetic acid for about 10 sec. and kept in 70 % alcohol for 15 min.; after that they were hydrolysed in 1N HCl at 60 °C for 12 min. (for further information see HAUSCHTECK-JUNGEN, 1970). To get reproducible DNA values, the brain had to be incubated in Schiff's reagent [Pararosanilin (Base) MERCK] for two or three hours. We used the plug method and a wavelength of 570 nm. Two different instruments, one constructed by Prof. F. RUCH, Botanisches Institut der ETH Zürich, the other the Zeiss Mikroskop-Photometer 01, gave the same results.

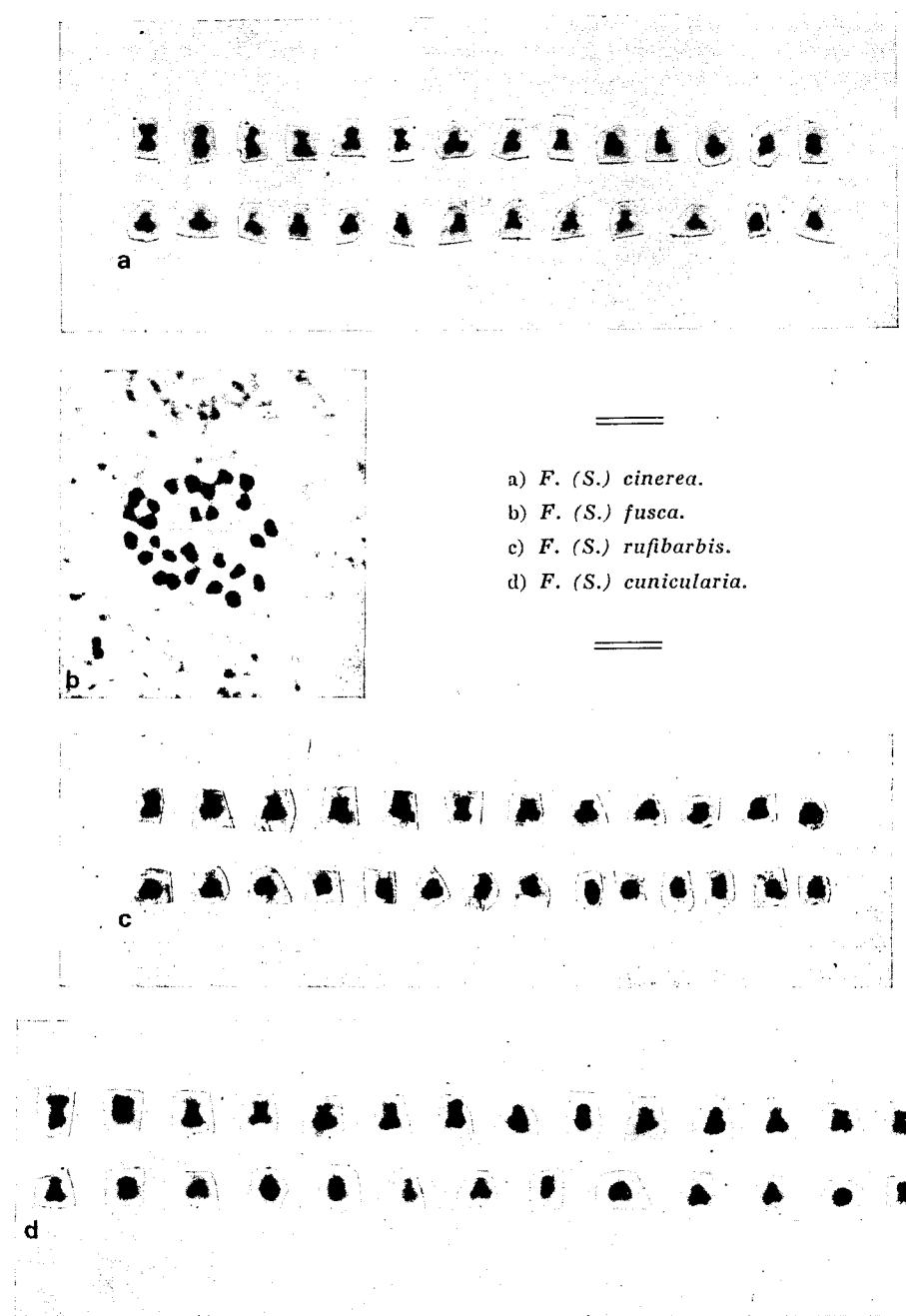
RESULTS

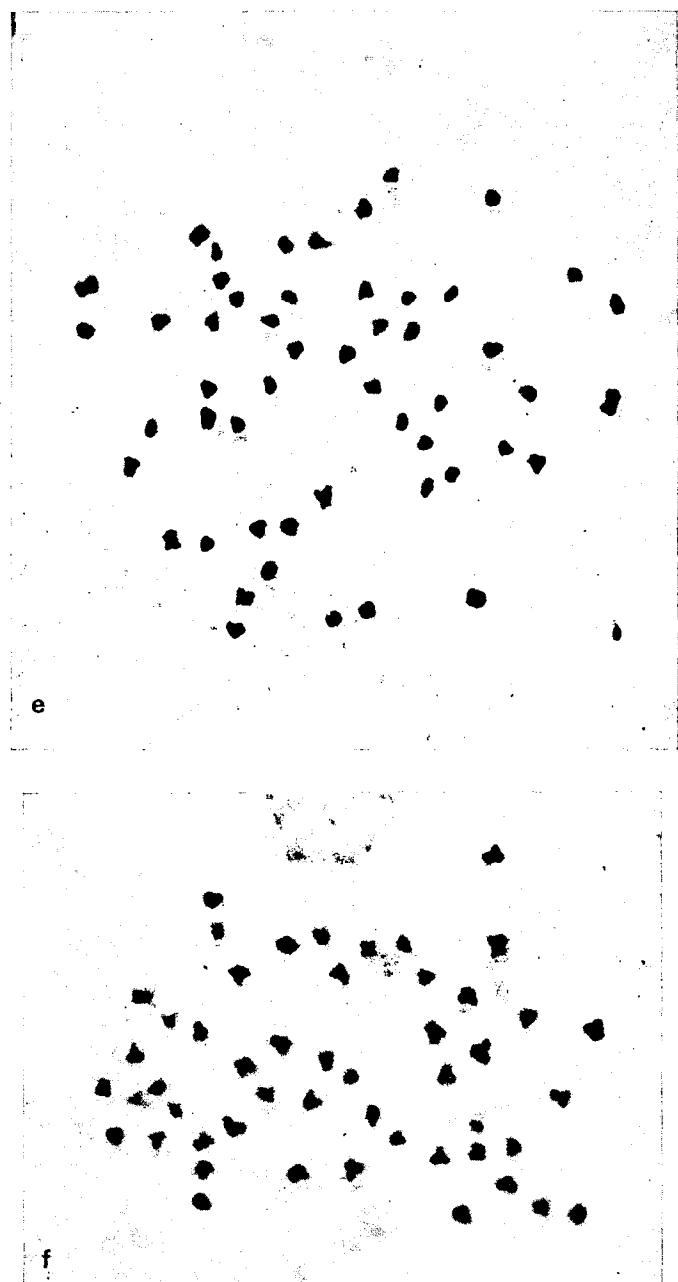
Karyotype analysis.

In Table I, the species together with their chromosome numbers are listed, including localities of collection and number of investigated nests. All karyotypes look more or less similar. There are slight differences between the length of chromosomes in a set. The ratio between the largest and the smallest chromosome is about 2:1.

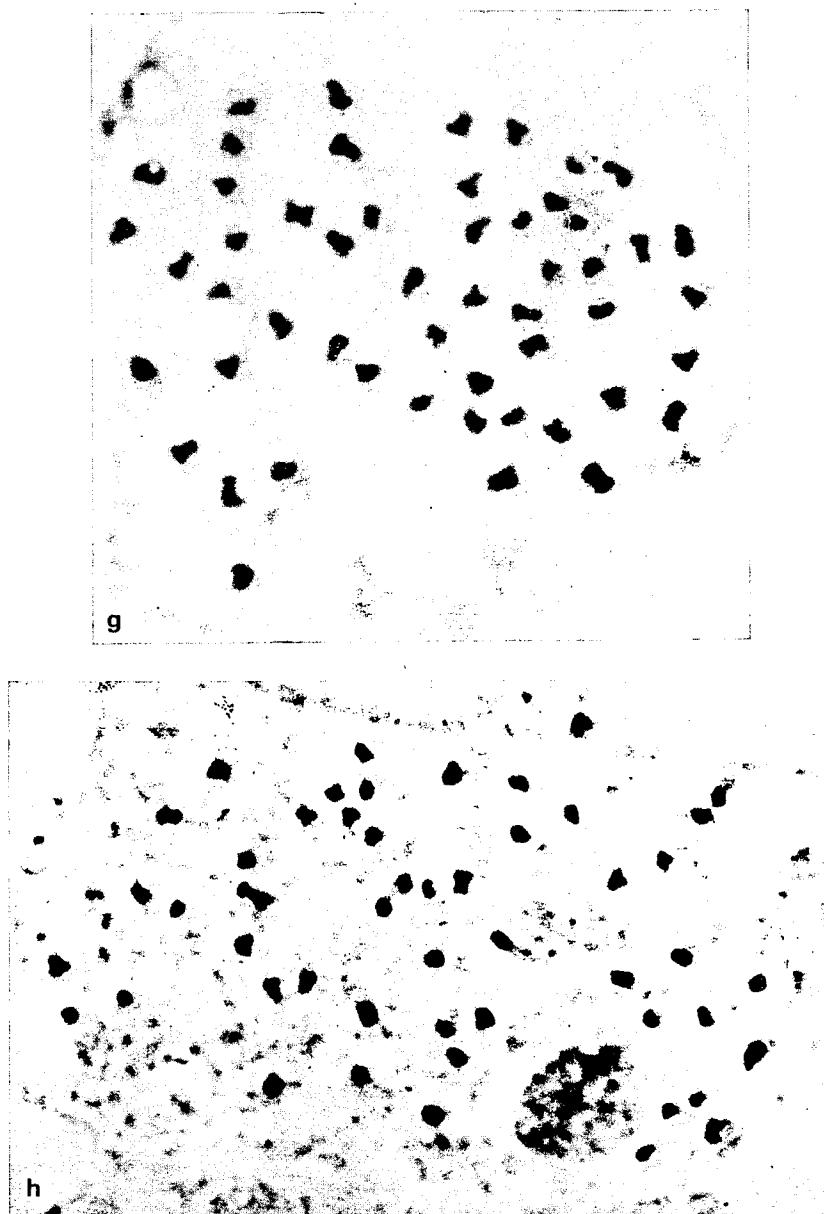
The following description will show that differences in the positions of the centromeres are between species of a subgenus but not between subgenera. In *F. (S.) rufibarbis*, *F. (S.) cunicularia*, *F. (S.) cinerea*, *F. (F.) polyctena*, and *F. (F.) truncorum* one of the first chromosomes (or possibly the first chromosome itself) is mediocentric. In *F. (S.) pratensis*, *F. (F.) rufa*, *F. (C.) exsecta*, and *F. (S.) picea* the corresponding chromosome is slightly submediocentric. The first four or five chromosomes of all karyotypes are similar in size. Among the ten largest chromosomes, one is acrocentric whereas the others are medio- or submediocentric. In all karyotypes analysed we found the smallest chromosomes to be subtelo- or submediocentric. The length of these chromosomes are in the range comparable to the microchromosomes of birds. Therefore, in the smallest chromosomes, the position of the centromere is obscure.

In *F. (S.) cinerea*, *F. (S.) cunicularia*, *F. (F.) pratensis* and *F. (F.) rufa* we observed the same type of faded short arms which IMAI (1969) showed in *F. (R.) sanguinea*. In *F. (F.) polyctena* and *F. (F.) truncorum* there are chromosomes with satellites (fig. 1).

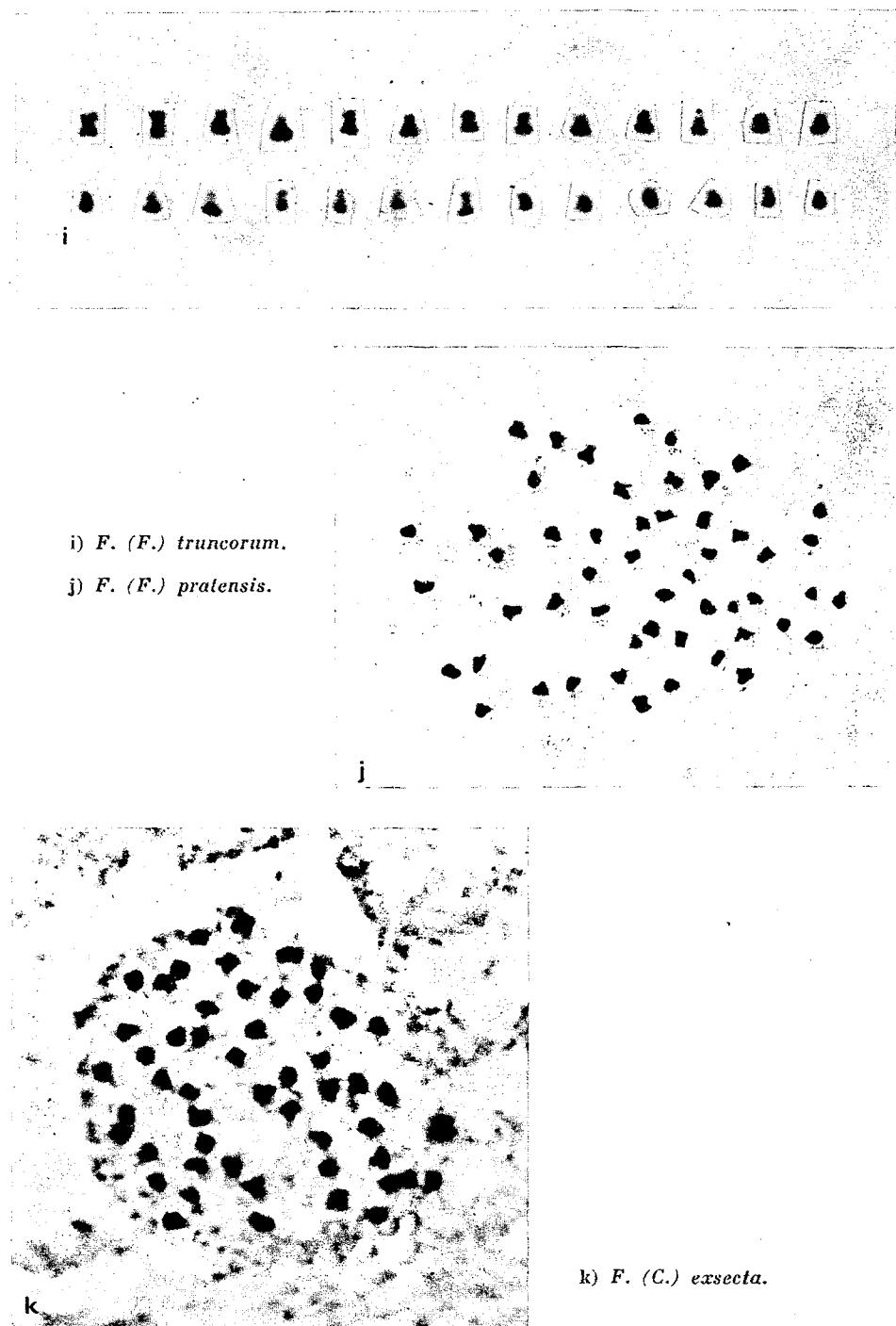
FIG. 1 *a-l.* — Metaphase plates and karyotypes. 2,800 \times .FIG. 1 *a-l.* — Metaphaseplatten und Karyotypen. 2 800 \times .



e) *F. (S.) picea*; f) *F. (F.) rufa*.



g) *F. (F.) polycrena*; h) *F. (F.) lugubris*.



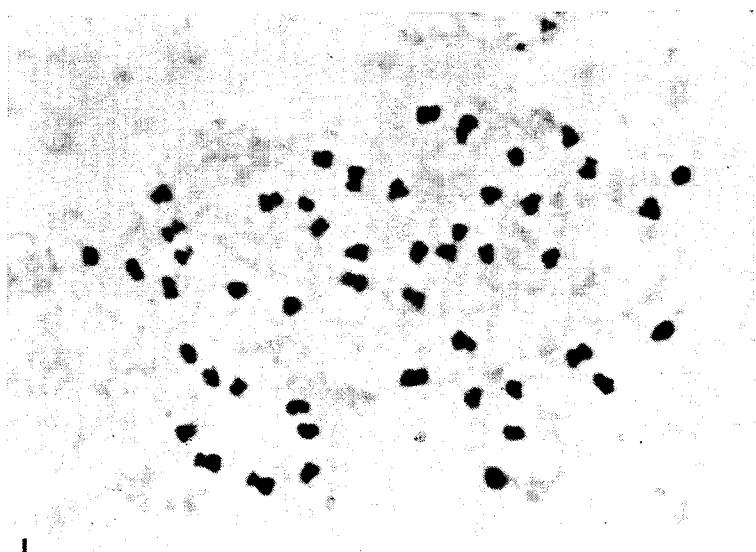
1) *F. (R.) sanguinea.*

TABLE I. — Chromosome numbers in the genus *Formica*. CH = Switzerland, F = France, D = Western Germany. All localities where no number of nests is added one nest had been investigated.

TABLE I. — Chromosomenzahlen der Gattung *Formica*. CH = Schweiz, F = Frankreich, D = West-Deutschland. Von Fundorten, bei denen die Anzahl untersuchter Nester nicht angegeben ist, wurde je ein Nest untersucht.

Genus Formica	Locality	2n	♀♂♂ investigated	Number of diploid or haploid nuclei
<i>F. (Serviformica) cinerea</i> Mayr....	Zürich (CH) (3 nests)	54	♀♀♂	>10
<i>F. (S.) fusca</i> L.	Sur En (CH)	54	♂	>10
<i>F. (S.) lemani</i> Bondr.	Safiental (CH)	54	♀	1
<i>F. (S.) rufibarbis</i> Fabr.	Schweiz/Switzerland Weiningen (CH)	54	♂	>10
<i>F. (S.) gagates</i> Latr.	Ain (F)	54	♀♂	>10
<i>F. (S.) cunicularia</i> Latr.	Jugoslavien/Yugoslavia	54	♂	>10
<i>F. (F.) picea</i> Nyl.	Ain (F)	54	♂	>10
	Illnau (CH) (4 nests)	52	♀	>10
<i>F. (Formica) rufa</i> L.	Winterthur (CH)	52	♀	>10
<i>F. (F.) polyctena</i> Foerst.	Andelfingen (CH)	52	♀	>10
<i>F. (F.) lugubris</i> Zett.	Schwägalp (CH)	52	♀	>10
<i>F. (F.) truncorum</i> F.	Fellers (CH)	52	♂	>10
<i>F. (F.) pratensis</i> Retz.	Ossingen (CH)	52	♀	>10
	Weiningen (CH)	52	♀	>10
<i>F. (Coptoformica) exsecta</i> Nyl....	Zermatt (CH) (2 nests)	52	♀	>10
<i>F. (Raptiformica) sanguinea</i> Latr..	Pfynwald (CH)	52	♀	>10
	Malix (CH)	52	♀♂	>10
	Zürich (CH)	52	♀♂	>10

Relative DNA amount per nucleus.

The relative DNA values per nucleus of four species belonging to three subgenera are shown in Table II. After testing for homoscedasticity using Bartlett's test, the four means were compared by means of a single classification analysis of variance. The following independent hypothesis were formulated

TABLE II. — Relative DNA values and diameters of single nuclei of four *Formica* species and of *Lasius (Dendrolasius) fuliginosus*. The DNA values are corrected with the nucleus volume after RUTHMANN (1966). For statistical purpose the numbers indicated in brackets are valid, because in each of these individuals the same number of nuclei were measured.

TABLE II. — Relativer DNS-Wert und Kerndurchmesser von vier *Formica*-Arten und von *Lasius (Dendrolasius) fuliginosus*. Die DNS-Werte sind korrigiert mit dem Kernvolumen nach RUTHMANN (1966). Für die statistischen Berechnungen wurde nur die in Klammern gesetzte Anzahl Tiere benutzt, weil bei jedem von diesen die gleiche Anzahl Kerne gemessen wurde.

Species	Rel. DNA amount/nucleus \pm standard deviation	Number of investigated		Mean diameter of 100 nuclei in μm \pm standard deviation
		animals	nuclei	
<i>Formica (Serviformica) fusca</i>	0.4135 \pm 0.0480	6 (5)	81	0.944 \pm 0.131
<i>Formica (Formica) polyctena</i>	0.4625 \pm 0.0656	31 (11)	234	1.013 \pm 0.099
<i>Formica (Formica) lugubris</i>	0.5092 \pm 0.0850	12 (7)	408	1.046 \pm 0.102
<i>Formica (Raptiformica) sanguinea</i> .	0.5433 \pm 0.0735	8 (5)	95	0.971 \pm 0.132
<i>Lasius (Dendrolasius) fuliginosus</i> .	0.3417 \pm 0.0442	23	432	0.962 \pm 0.064

TABLE III. — Comparisons among the means of the DNA values of *Formica* species. Results of the ANOVA, single classification, with sum of squares decomposed into planned comparisons. SS = sum of squares, df = degree of freedoms, MS = mean squares.

TABLE III. — Vergleich der DNS-Mittelwerte der *Formica*-Arten. Ergebnisse einer Varianzanalyse mit einfacher Klassifikation. Die zu vergleichenden Gruppen wurden im voraus bestimmt. SS = Summenquadrate, df = Anzahl Freiheitsgrade, MS = Durchschnittsquadrate.

Source of variance	SS	df	MS	F
Between species	0.0542	3	0.0180	4.8016 (*)
<i>Serviformica</i> vs.				
<i>Formica</i> + <i>Raptiformica</i>	0.0310	1	0.0310	8.2428 (*)
<i>Formica</i> vs. <i>Raptiformica</i>	0.0085	1	0.0085	2.2743
among <i>Formica</i>	0.0146	1	0.0146	3.8875
Within species	0.1091	29	0.0037	

(*) Significant at the 1 % level.

(*) Signifikanz auf dem 1 % Niveau.

a priori : 1) Differences exist between the subgenus *Serviformica* on the one hand and the more evolved subgenera *Formica* and *Raptiformica* on the other hand. 2) Differences exist between the subgenera *Formica* and *Raptiformica*. 3) Differences exist within the subgenus *Formica*. The ANOVA table (Table II) presents the data including the between species sum of squares decomposed into the planned comparisons. The resulting F-values « between species » and « *Serviformica* versus *Formica* and *Raptiformica* » are significant at the 1 % level. Evidently the difference of *Serviformica* vs. other subgenera contributes the greatest amount to the total effect of heterogeneity between the four species. The differences between the four species were tested in the *a posteriori* test of Student-Newmann-Keuls (SOKAL and ROHLF, 1969). The result is tabulated in Table III. There exists no obvious gap between *F. (S.) fusca* and the other species; however, the tendency is the same as in the *a priori* test.

DISCUSSION

IMAI and YOSIDA (1964) and IMAI (1966, 1969) reported chromosome numbers of six *Formica* species, HUNG (1969) of four. All species had the diploid chromosome numbers of 52 or 54. In the present paper the chromosome numbers of *F. (S.) fusca*, *F. (S.) picea*, *F. (F.) truncorum* and *F. (R.) sanguinea* were confirmed. In addition to the chromosome number, the karyotype of *F. (F.) truncorum* is presented. HUNG (1969) published the mitotic figures of four species. In all mitoses the majority of the chromosomes seems to be medio- or submedio-centric. The only karyotype analysis done by IMAI (1969) was on *F. (R.) sanguinea*. He claimed the karyotype as exceptional because it is missing acrocentric chromosomes, which he said are normally present in karyotypes with large chromosome numbers. He therefore assumed that this genus has derived from an ancestor with lower chromosome number by polyploidisation.

One of IMAI's argument for the existence of polyploidy in *Formica* is his statement that the karyotype of *Formica* might consist of two similar sets of chromosomes. In our material, and to a certain extent also in *F. (R.) sanguinea* (IMAI, 1969), it is hardly possible to identify individual chromosomes which are smaller than about chromosome number ten. Therefore it seems to us nearly impossible to decide whether there are two identical sets of chromosomes in the haploid set of *Formica*. We can confirm that in many species the first chromosome is a well defined one as in *F. (R.) sanguinea*.

IMAI (1969) speaks of so-called « partial polyploidy », by which he means multiple trisomy. Normally, partial polyploidy seems to be a very minor evolutionary factor, for karyotype analysis in animals shows that trisomy leads to strong malformations of the carrier or is even lethal. The same is true for large duplications and deficiencies.

The high chromosome number of *Formica* is correlated with a high DNA

value, higher than in species with lower chromosome numbers. The increased amount of DNA per nucleus might originate from gene amplification rather than from polyploidisation. Species with lower chromosome numbers than *Formica*, as, for instance, *Lasius (Dendrolasius) fuliginosus* with $2n = 28$, have more DNA per nucleus than expected when compared to *Formica*.

Species differentiation in *Formica* is generally not conducted by changes in chromosome number, with one exception, namely, *F. (S.) picea*. It is the only species of the subgenus *Serviformica* with diploid 52 instead of 54 chromosomes. Because of the similarity of the chromosomes it is impossible to analyse whether a Robertsonian type of translocation is involved. On the level of the subgenera we find two groups : *Serviformica* with $2n = 54$ and the other three subgenera with $2n = 52$ chromosomes. Several arguments indicate that *Serviformica* is the most primitive subgenus within the genus *Formica*. For example, there are no species with social parasitic phase in starting a new colony (KUTTER, 1969).

In contrast, queens of most of the species of the other subgenera use, at least optionally, colonies of *Serviformica* species as hosts. Furthermore, at least some *Serviformica* species contain endosymbiotic microorganisms (BUCHNER, 1953; JUNGEN, 1968) while in the subgenera *Formica* and *Raptiformica* only remnants of a former endosymbiosis are found (BUCHNER, 1953). Another point is that *Serviformica* shows the lowest DNA amount per nucleus. Therefore it is possible that the evolution of the genus *Formica* began with a « *Serviformica* stage » and that the other subgenera descended monophyletically from a common ancestor. In view of the fact that there is little variation in the chromosome numbers, it seems unlikely that the chromosome numbers of the other three subgenera have developed by parallel evolution. However, the opposite hypothesis, that the *Serviformica* species excluding *F. (S.) picea* have branched from the main stock of species with $2n = 52$ chromosomes, cannot be excluded even if it looks less probable than the former hypothesis.

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