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Biochemical genetic differentiation among seed chalcid species of genus *Megastigmus* (Hymenoptera: Torymidae)

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Abstract. The degree of genetic variability and the taxonomic status of adults of 14 seed chalcid species of the genus *Megastigmus* was analyzed using electrophoresis on horizontal starch gel. Variability of chalcid populations with host tree was observed in 21 host species. A total of 13 enzyme loci were considered. Seven of the loci were found to be polymorphic. The electrophoretic data strongly supported the adaptation of each chalcid species to a limited number of congeneric hosts, and confirmed the morphologically-based taxonomy of the genus. The resulting dendrogram separated the chalcid species into three distinct groups, infesting 1) Pinaceae spp., 2) Cupressaceae spp., and 3) Angiosperm spp., respectively. The highest level of overall genetic similarity was observed among the chalcid populations infesting conifers of the genera *Pseudotsuga* and *Abies*. The genetic identity values observed among populations infesting 5 different *Abies* species tended to reflect the occurrence of conspecific populations rather than that of distinct chalcid species. Genetic identity was similarly important among the chalcid species infesting seeds of Cupressaceae. By contrast, a large genetic distance was observed between two seed chalcids attacking a same host, *Rosa montana*.
Key words. Seed chalcids; *Megastigmus*; hymenoptera; isozymes; phylogeny.

The correct identification of species is an important prerequisite for biological investigations[1]. Although many groups of phytophagous insects are restricted to a narrow set of host taxa[2], the formation of host races or sibling species among specialized phytophagous insects may originate in an adaptative response to a plant species different from the primary host. Zwölfer and Herbst[11] pointed out that strongly specific insect-plant associations are the most likely candidates for parallel diversification and hence for long-term, pairwise, coevolution. From this point of view, the evolutionary relationships between trees and specialized seed-eating insects of the genus *Megastigmus* (Hymenoptera: Torymidae) are an interesting subject of study. *Megastigmus* seed chalcids have an almost world-wide distribution[12]. Turgeon et al.[13] listed 45 species attacking exclusively the seeds of conifers. A few others are specialized on angiosperm seeds, essentially Rosaceae and Anacardiaceae[12]. Parasitic species probably belong to a different genus[14].

The biology and taxonomy of seed chalcids are well documented in Europe[15–20], but the degree of host specificity remains unclear. In western Europe, Roques[21] observed that each native *Megastigmus* species (except seed chalcids attacking *Juniperus* spp.) infests only one indigenous conifer, but this situation was assumed to have resulted from the survival of only one species per genus in most of the conifer genera (e.g., *Abies*, *Larix*, *Picea*) following the Quaternary glaciations. Moreover, most of the European species of *Megastigmus* seem to be capable of colonizing seeds of exotic conifers introduced to Europe when the introduced conifer is congeneric with the native host[22]. The host range of seed chalcids attacking angiosperm seeds appears to be wider, and may be related to a larger specific diversification in the host genera, e.g. *Rosa*[23]. As a result of the increasing world trade in seeds, additional seed chalcids mostly originating from North America have been introduced to Europe along with their original hosts, and some of them have shifted to European conifers[19, 22].

The present economic importance of seed chalcids with respect to the protection of genetically-improved seeds in tree seed orchards gives rise to a need to understand whether the colonization of different tree species is a result of the plasticity of the insects, or whether it is from populations already adapted to each host tree. The study of electrophoretically detectable enzyme polymorphism has proven to be a valuable tool for the identification of sibling and cryptic species, and also for estimating genetic divergence between closely related forms[24]. The genetics of seed chalcids has been little studied, apart from some preliminary work on a cedar seed chalcid[25]. Our study therefore aimed at estimating and comparing the levels of genetic differentiation among the *Megastigmus* species observed in Europe and Asia minor in relation to host tree.

Materials and methods

Insect collection. Seed samples were collected in 1992–1993 on 21 tree and shrub species known to be attacked by *Megastigmus* seed chalcids (table 1). Both native and

Table 1. *Megastigmus* species (abbreviations used), host tree, collection sites and total number of insects studied.

Host tree species	Presumed insect sp.	Native or introduced	Collection sites	Coordinates	Abbr.	Total of individuals
Pseudotsuga menziesii	*M. spermotrophus*	i	Lavercantière	44°52′N, 2°02′E	sp	99
Pseudotsuga guinieri	*M. spermotrophus*	i	Les Barres	47°50′N, 2°45′E	sp′	1
Pseudotsuga japonica	*M. spermotrophus*	i	Angers	47°28′N, 0°36′W	sp*	19
Larix gmelini	*M. pictus*	n	Les Barres	47°50′N, 2°45′E	pic	5
Picea orientalis	*M. atedius*	i	Les Barres	47°50′N, 2°45′E	ate	6
Abies nordmanniana	*M. suspectus*	n	Les Barres	47°50′N, 2°45′E	su1	9
Abies alba	*M. suspectus*	n	Les Barres	47°50′N, 2°45′E	su2	12
Abies nebrodensis	*M. suspectus*	n	Les Barres	47°50′N, 2°45′E	su3	2
Abies pinsapo	*M. suspectus*	n	Les Barres	47°50′N, 2°45′E	su4	43
Abies numidica	*M. suspectus*	n	Les Barres	47°50′N, 2°45′E	su5	15
Abies pardei	*M. suspectus*	n	Les Barres	47°50′N, 2°45′E	su6	4
Cedrus atlantica	*M. pinsapinis*	n	Les Barres	47°50′N, 2°45′E	pin	8
Cedrus libani	*M. schimitscheki*	n	Turkey	37°30′N, 35°00′E	sch	3
Abies nobilis	*M. pinus*	i	Vielsam	50°20′N, 5°55′E	pi	98
Rosa montana	*M. aculeatus*	n	Briançon	44°51′N, 6°36′E	acu	69
Rosa montana	*M. rosae*	n	Névache	45°01′N, 6°38′E	ros	6
Juniperus communis	*M. bipunctatus*	n	Névache	45°01′N, 6°38′E	bip	14
Juniperus pingii	*M. pingii*	n	Lijiang	27°01′N,100°09′E	sab	4
Juniperus phoenicea	*M. amicorum*	n	Ericeira	38°58′N, 9°26′W	ami	14
Juniperus oxycedrus	*M. amicorum*	n	Eyguières	43°43′N, 5°02′E	am*	1
Pistacia terebinthus	*M. pistaciae*	n	Cabo Espichel	38°25′N, 9°13′W	pis	29
Cupressus sempervirens	*M. wachtli*	n	Kavalla	24°22′N, 40°56′E	wa	35

i = seed collected in the introduced range of the insect species.
n = seed collected in the native range of the insect species.

introduced tree species were considered. Seeds were collected mainly in the arboretum of "Les Barres", central France, but also in natural and artificial stands of southern France, Belgium, Greece, Portugal, and Turkey (table 1). In addition, we were supplied with infested juniper seeds originating from southern China. The collection of female reproductive structures (i.e., seed cones, juniper galbulae, pistachio nuts and rose hips) was conducted at the time of seed maturity. After a limited amount of drying, seeds were separated from other structures, and X-rayed in order to identify those that were chalcid-infested. Infested seeds were kept under outdoor conditions in separate containers until adult chalcids emerged. As soon as they emerged, adults were sexed, identified to the species level, and frozen at −80°C until electrophoretic study.

A total of 496 adults, representing 13 of the 19 *Megastigmus* seed chalcid species presently recorded in Europe, North Africa and Asia Minor (Roques, unpubl. data), were thus obtained. Individuals of 9 species came from the species' native range: 1) *M. pictus* Förster, observed on *Larix* spp. in Eurasia[20]; 2) *M. suspectus* Borries, recorded on most of the *Abies* species all over Europe to the Caucasus[20, 26, 27]; 3) *M. bipunctatus* Swederus, observed on the alpine species of *Juniperus*[20]; 4) *M. amicorum* Bouček, observed on the Mediterranean species of *Juniperus*[20]; 5) *M. wachtli* Seitner, observed on the Mediterranean cypress, *Cupressus sempervirens* L.[28]; 6) *M. schimitscheki* Novitzky, recorded on *Cedrus libani* Loudon and *C. brevifolia* (Hook.f.) Henry in the eastern part of the Mediterranean basin[29]; 7–8) *M. aculeatus* Swederus and *M. rosae* Bouček, recorded on *Rosa* seeds[19]; and, 9) *M. pistaciae* Walker, a specialist of *Pistacia* seeds[27]. Individuals of 4 other species were collected in areas of introduction: 10) *M. spermotrophus* Wachtlus, which was introduced from north America to Europe along with Douglas-fir (*Pseudotsuga menziesii* Mirb.), and shifted to most of the exotic species of *Pseudotsuga*[30]; 11) *M. pinus* Parfitt, a specialist of north American species of *Abies* that shifted to some native Mediterranean firs such as *A. pinsapo* Boissier[20]; 12) *M. atedius* Walker, which attacks seeds of both *Pinus strobus* L. and *Picea* spp. in north America[31] but shifted to *Picea orientalis* L. in Europe[20]; and 13) *M. pinsapinis* Hoffmeyer, probably originating from north Africa on *Cedrus atlantica* Manetti, that attacks seeds of both cedars and European firs, especially *A. pinsapo*[25]. In addition, we considered 14) *M. pingii* Roques et Sun, a new species found on *Juniperus pingii* Cheng et Wang in Yunnan province of China[52]. Most of the species were represented by a single population except *M. suspectus* (populations from 5 *Abies* species), *M. spermotrophus* (populations from 3 *Pseudotsuga* species) and *M. amicorum* (populations from 2 juniper species). The size of the population samples ranged from 1 to 99 individuals because chalcid damage varied widely with both species and location. Many populations were originated from Les Barres (France). In the case of rare species, only a small number of individuals could be obtained. Because of the

Table 2. Enzyme assayed and number of loci resolved for each.

Enzyme Systems	Code	Loci	Buffer*
α-Glycerophosphate dehydrogenase	αGPD	3	C
Glucose phosphate isomerase	GPI	1	B
Hydroxibutyrate dehydrogenase	HBDH	1	A
Isocitrate dehydrogenase	IDH	1	A
Lactate dehydrogenase	LDH	1	B
Malic enzyme	ME	1	B
Phosphoglucomutase	PGM	1	A
Pyruvate kinase	PK	1	A
Aminopeptidase (Glycil-L-leucine)	AP	1	A
Superoxyde dismutase	SOD	1	C
Arginine phosphokinase	APK	1	A

*A = Continuous Tris/citrate buffer, pH 8.
B = Phosphate buffer, pH 6,3.
C = Discontinuous Tris/citrate buffer, pH 8,2.

great systematic relevance of these species, we decided to not remove them from the analysis, but the results should be taken with caution.

Laboratory procedure. Individual chalcids were homogenized in 50 µl of Tris-HCl buffer 0.01 M pH 8, immediately before the electrophoretic run. The crude homogenate was absorbed onto a 10 × 4 mm Whatman filter paper (n°3). Electrophoresis was then performed on horizontal starch gel using 10% Connaught starch and 250 ml gel buffer. Representatives of the 14 *Megastigmus* species were loaded together in the same gel plate for comparison. Pairs of individuals from each species or population were spaced to maximize the number of direct comparisons between all species, and specimens of *M. spermotrophus* were included on each gel as standards. This arrangement controlled for any irregularities on the gel and any day-to-day variation in overall migration distance, and thus overcame the possible effects of inconsistencies between gels.

The gels were run for 5 h at 60 mA and 150 V for a Tris/citrate buffer system[32], at 90 mA and 110 V for a Phosphate buffer system[33], and for 3.5 h at 90 mA and 200 V for a Poulik buffer system[34]. Then, gels were sliced horizontally and each of the sheets was stained for a different enzyme. Eleven enzymes (13 loci) were assayed in each of the 14 chalcid species (table 2). No interpretable gel was obtained for MDH, PEP and 6-PGDH, where variations were observed but could not be clearly scored. Stain recipes were adapted from those detailed in[32, 33, 35, 36, 37].

Because of the haplodiploid system of sex determination in Hymenoptera, each individual was scored according to sex (male: 1 ; female: 2). Loci and alleles were coded according to relative mobility. The most common band in *M. spermotrophus* was taken as a standard. All calculations employed the BIOSYS-1 computer program of Swofford and Selander[38]. Because of complexities introduced by the haploid sex determination, statistical analyses used allele frequency data. Nei's[39] and Rogers'[40] measures of genetic distance and identity were chosen for inter- and intra-specific comparisons. In a few cases, some loci were not resolved in the smallest populations. To include such populations in a computer calculation of genetic distances, we assigned the most conservative allelic designation to one individual of these populations, following Woods and Guttmann[41]. Since the choice of alleles is conservative, any distortion tends to underestimate genetic distances. Nei's genetic identity values (I) and Rogers' genetic distance (D) were finally used to obtain dendrograms using the complete linkage clustering method[42] and the distance Wagner procedure[43], respectively.

Results

Table 3 presents the results of the electrophoretic analysis. Only one locus (APK) among the 13 analysed was invariant across all populations studied. Numerous loci were invariant within a single species and showed an allele unique to that species, or nearly so (Ap^B-pis, Ap^D-sch, Ap^E-ros, Ap^F-pic, $Hbdh^C$-pic and acu, Ldh^D-pi, Ldh^F-acu, Pk^C-acu and ros). In several cases, different species were fixed for alternate alleles (e.g., AP, GPD2, GPD3, PK, SOD). The allelic frequencies allowed three groups of insect populations to be separated, which corresponded to different host taxa: 1) Pinaceae (populations 1 to 14), 2) Cupressaceae (populations 15 to 19), and 3) Angiosperms (populations 20 to 22).

1) Pinaceae. Seed chalcids from *Pseudotsuga* spp. clearly differed in having no enzyme activity (null allele) at the GPD3 locus. These conspecific populations were very similar. The frequency differences observed at some loci were probably due to the size of the samples. The chalcids originating from *Abies* seeds constituted the most variable and complex group. *M. suspectus* from *A. nordmanniana* was fixed for an alternative allele (Ap^C) while chalcids from other *Abies* spp. were not. *M. pinus* from *A. nobilis* was distinguished by the fixation on Ldh^D allele. Five loci were polymorphic in most of the *Abies* seed chalcids (HBDH, IDH, ME, GPI, PGM).

Seed chalcids from *Cedrus* spp. (pin, sch) showed alternative fixed alleles at loci AP, IDH1, LDH and ME, and clearly differed from chalcids infesting *Abies* spp.

M. pictus from *Larix* was polymorphic for only one locus (GPI). It showed 2 alleles, B and E, in equal number, since we observed only heterozygous females at this locus. This species usually reproduces by thelytokous parthenogenesis. *M. atedius* from *Picea* was fixed for all the observed alleles. However, owing to the limited size of the studied populations, the results regarding the two last species must be viewed with caution.

2) Cupressaceae. The seed chalcids attacking species of *Juniperus* and *Cupressus* showed similar alleles in most

Table 3. Allele frequencies of enzyme loci and mean expected heterozygosity in the studied *Megastigmus* species.

Abbr.	sp	sp'	sp*	pic	ate	su1	su2	su3	su4	su5	su6	pi	pin	sch	bip	sab	ami	am*	wa	pis	acu	ros
N	99	01	19	05	06	09	12	02	43	15	04	98	08	03	14	04	14	01	35	29	69	06
AP-A	1.00	1.00	1.00	*	1.00	*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	*	1.00	1.00	1.00	1.00	1.00	*	*	*
AP-B	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00	*	*
AP-C	*	*	*	*	*	1.00	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00	*
AP-D	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00	*	*	*	*	*	*	*	*
AP-E	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00
AP-F	*	*	*	1.00	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
APK-A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
GPD1-A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	*	1.00	1.00	1.00	1.00	1.00	1.00
GPD1-B	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.50	*	*	*	*	*	*
GPD1-C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0.50	*	*	*	*	*	*
GPD2-A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	*	1.00a	*	*	*	1.00	*	1.00
GPD2-B	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00	*	1.00	1.00	1.00	*	1.00	*
GPD3-A	1.00a	1.00a	1.00a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00a	1.00	1.00	1.00	1.00	*	1.00
GPD3-B	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00	*
HBDH-A	1.00	1.00	1.00	*	1.00	0.55	0.80	1.00	0.97	1.00	1.00	1.00	1.00	1.00	*	1.00	1.00	1.00	1.00	1.00	*	1.00
HBDH-B	*	*	*	*	*	0.45	0.20	*	0.03	*	*	*	*	*	1.00	*	*	*	*	*	*	*
HBDH-C	*	*	*	1.00	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00	*
IDH1-A	1.00	1.00	1.00	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
IDH1-B	*	*	*	1.00	*	*	0.57	*	0.12	*	*	1.00	1.00	*	*	*	*	*	*	1.00	*	1.00
IDH1-C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00	1.00	1.00	1.00	1.00	*	*	*
IDH1-D	*	*	*	*	1.00	1.00a	0.43	1.00a	0.88	1.00	1.00	*	*	*	*	*	*	*	*	*	1.00	*
IDH1-E	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00	*	*	*	*	*	*	*	*
LDH-A	1.00	1.00	1.00	1.00	1.00	*	*	*	*	*	*	*	*	1.00	1.00	1.00a	*	*	1.00	0.93	*	1.00
LDH-B	*	*	*	*	*	*	*	*	*	*	*	*	1.00	*	*	*	*	1.00	*	*	*	*
LDH-C	*	*	*	*	*	1.00a	1.00	1.00a	1.00	1.00	1.00	*	*	*	*	*	*	*	*	*	*	*
LDH-D	*	*	*	*	*	*	*	*	*	*	*	1.00	*	*	*	*	*	*	*	*	*	*
LDH-E	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00	*	*	0.07	*	*
LDH-F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00	*
ME-A	1.00	1.00	1.00	1.00	*	*	*	*	0.74	1.00	1.00	*	*	1.00	*	*	*	*	*	*	*	*
ME-B	*	*	*	*	1.00	1.00	1.00	1.00	0.26	*	*	1.00	1.00	*	*	*	*	*	*	1.00	1.00	1.00
ME-C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00	1.00	1.00	1.00	1.00	*	*	*
PK-A	1.00	1.00	1.00	1.00	1.00	1.00a	1.00	1.00a	1.00	1.00	1.00	1.00	1.00	1.00	1.00a	1.00a	*	*	*	*	*	*
PK-B	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00	1.00	1.00	1.00	*	*
PK-C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00	1.00
GPI-A	0.98	1.00	1.00	*	1.00	0.50	0.86	*	0.35	*	*	0.03	*	*	*	*	*	*	*	*	*	*
GPI-B	*	*	*	0.50	*	*	*	*	*	*	*	0.97	*	*	*	*	*	*	*	*	*	*
GPI-C	0.02	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
GPI-D	*	*	*	*	*	0.50	0.14	1.00	0.65	1.00	1.00	*	*	*	*	*	*	*	*	*	*	*
GPI-E	*	*	*	0.50	*	*	*	*	*	*	*	*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	*	*	1.00
GPI-F	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00	1.00	*
PGM-A	0.96	1.00	1.00	*	*	1.00	0.57	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
PGM-B	0.02	*	*	1.00	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00
PGM-C	0.02	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	*
PGM-D	*	*	*	*	1.00	*	0.43	1.00	1.00	1.00	1.00	1.00	1.00	1.00	*	*	*	*	*	*	*	*
SOD-A	1.00	1.00	1.00	1.00a	1.00a	1.00a	1.00	1.00a	1.00	1.00	1.00	1.00	1.00	1.00	1.00a	1.00a	*	*	*	*	*	*
SOD-B	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1.00	1.00	1.00	1.00	1.00	1.00
Mean He	.010	.000	.000	.043	.000	.081	.122	.000	.086	.000	.000	.005	.000	.000	.000	.044	.000	.000	.000	.010	.000	.000

Mean He = mean expected heterozygosity.
N = numbers of individuals examined.
a = when loci were not resolved in a population, the most conservative allele within the species or the genus was assigned.

cases. In *M. amicorum*, alleles were similar among populations from *J. phoenicea* and *J. oxycedrus*, except at the locus LDH where different alleles (Ldh^E-ami and Ldh^B-am*) were fixed for each population. However, the small size of the sample, especially in am* did not allow a conclusion to be drawn. *M. wachtli* was clearly separated by the diagnostic allele Ldh^A.

3) Angiosperms. Seed chalcids from *Rosa* (acu and ros) and *Pistacia* (pis) showed some allelic differences. The two chalcid species from *Rosa* were very different from each other, having only 5 loci with common alleles (Ap^K, $Gpd1^A$, Me^B, Pk^C and Sod^B). *M. rosae* seemed to be more closely related to *M. pistaciae* from *P. terebinthus*.

The mean expected heterozygosity values (table 3) varied among host forms from 0.000 to 0.122. The highest values were observed in *Abies* seed chalcids, especially from *A. alba* (0.122). Lower values (0.010, 0.043, 0.043) were observed in the *Megastigmus* species from *P. menziesii*, *L. gmelini* and *J. pingii*, respectively. The values (0.000) for certain populations should be viewed as provisional in the light of the small size of the samples.

The complete matrix of Nei's I and Rogers' D, that are not presented here, are available from the authors. Coefficients were used to obtain a UPGMA dendrogram from Nei's I (fig. 1), and a Wagner tree from Roger's D (fig. 2). The cophenetic correlations were

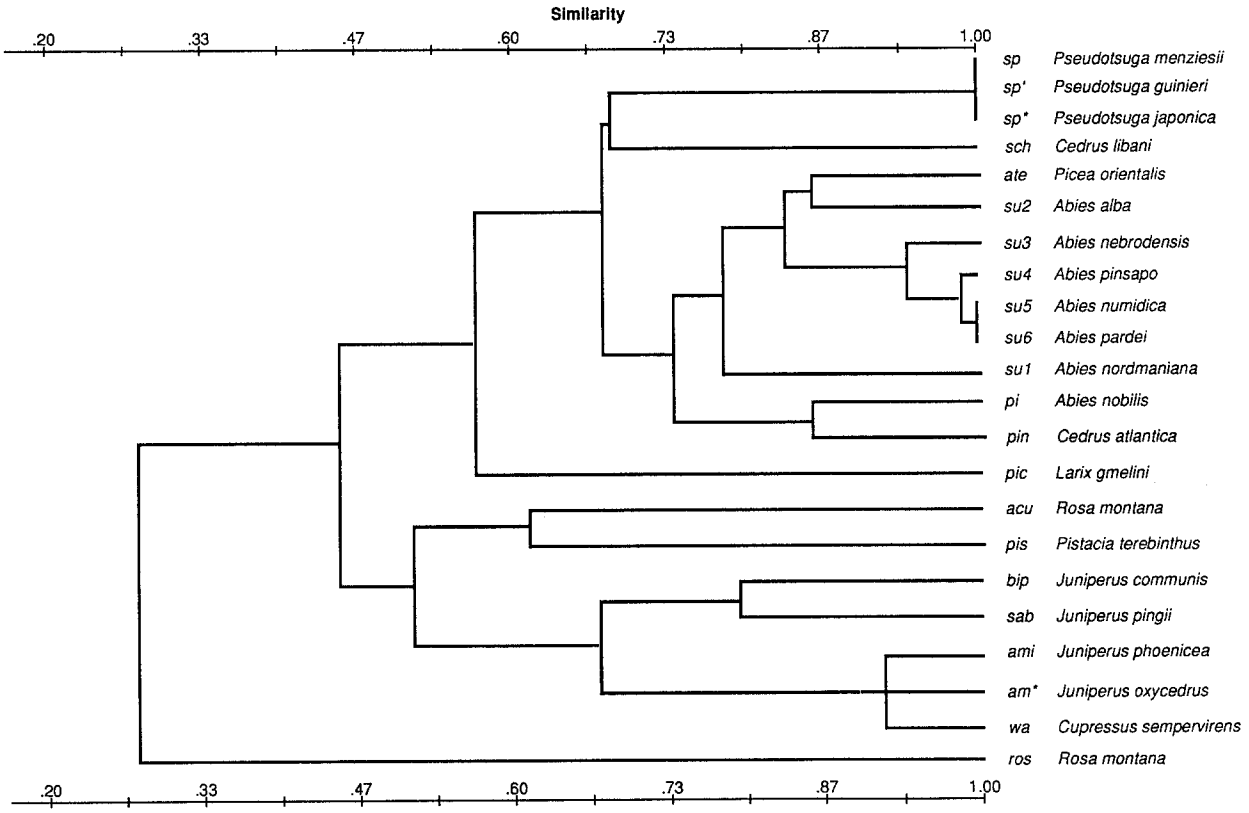


Figure 1. UPGMA dendrogram, based on Nei's identity, of populations of *Megastigmus*. The cophenetic correlation was 0.915 and the percent standard deviation of Fitch and Margoliash was 20.441.

0.915 and 0.866, respectively. The values indicated that the dendrograms fitted the matrix well. Both dendrograms clearly separated three sets of populations and species with respect to hosts. There were a few differences within groups. Chalcids from *Pinaceae* were well grouped according to host phylogeny in the Wagner tree, while the chalcid population from *Picea* clustered with that from *Abies alba* in UPGMA. *M. schimitscheki* from *Cedrus libani* was linked to *Pseudotsuga* chalcids in UPGMA, while it was separated from the other conifers in the Wagner tree. The Angiosperm chalcids grouped in the Wagner tree, while species from *Rosa montana* and *Pistacia terebinthus* clustered with those from *Cupressaceae* in UPGMA.

Discussion

Enzyme electrophoresis is a well-known method used to differentiate closely related species, even haplodiploid Hymenoptera that are less variable than other insects[44]. Few phytophagous Hymenoptera have been investigated[9, 10, 41, 45], and only one electrophoresis study dealt with *Megastigmus* species infesting cedars[25].

Megastigmus chalcids displayed rather low levels of genetic variation, as did most other aculeate hymenopterans[46]. The population of seed chalcids infesting *Abies alba* displayed a higher heterozygosity than other *Megastigmus* species. Many factors could explain this difference, such as introgressive hybridization between different races or species. The extremely low levels of enzyme-gene variation observed in *Megastigmus* seed chalcids indicated that each species and most populations were essentially fixed for one electromorph at each locus. As fixation for different allozymes at a locus is considered an important indicator of reproductive and genetic divergence[47], such a result suggests that reproductive isolation is well established among some of these groups, at least.

The genetic divergence observed within the genus *Megastigmus* also suggested that populations observed on different hosts may frequently differentiate and may undergo speciation. Similar results have been found in other phytophagous hymenopterans where allozyme data clearly documents the genetic differentiation of populations associated with different host-tree species[9, 10].

Populations of seed chalcids infesting most of the conifers of the Abietaceae family (i.e., *Abies*, *Cedrus*, and *Pseudotsuga*) were clearly separated from those infesting conifers of the Cupressaceae family (*Cupressus*, *Juniperus*), as well as from most of these infesting angiosperms (*Pistacia*, *Rosa*) (fig. 2). The genetic iden-

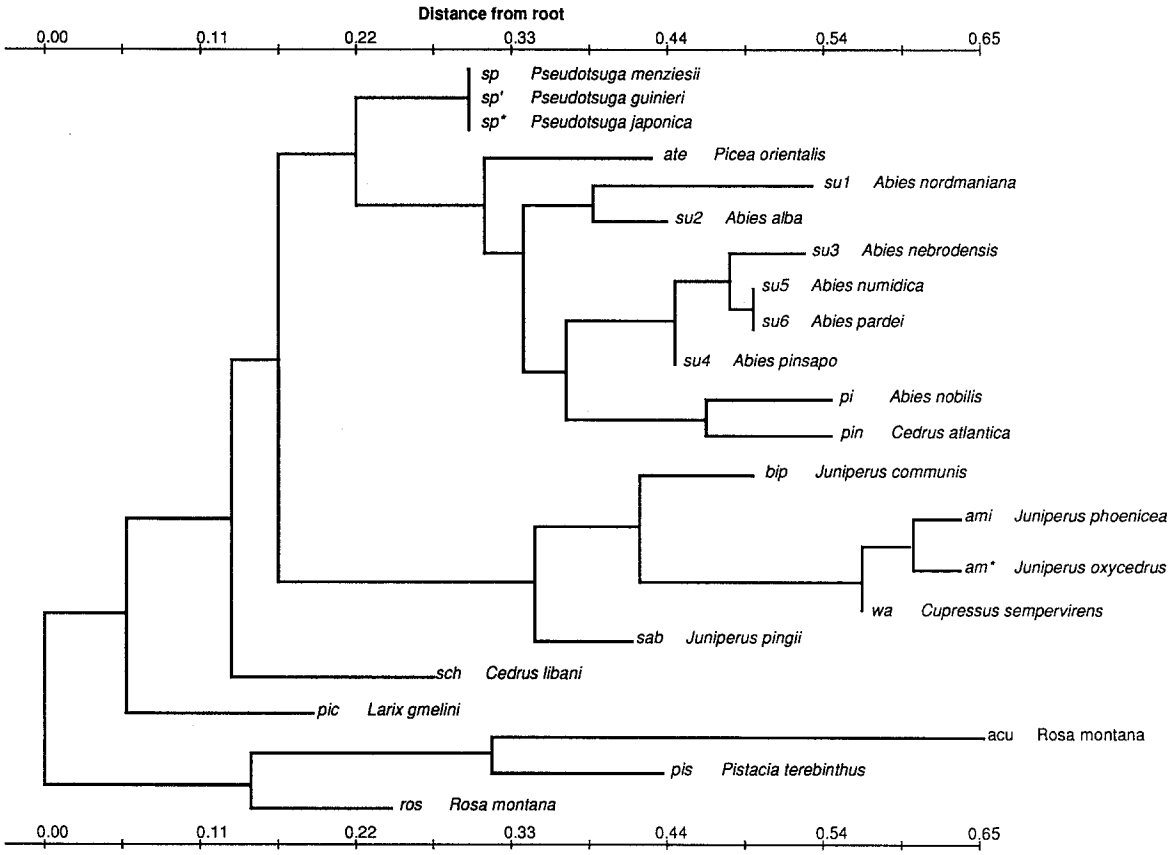
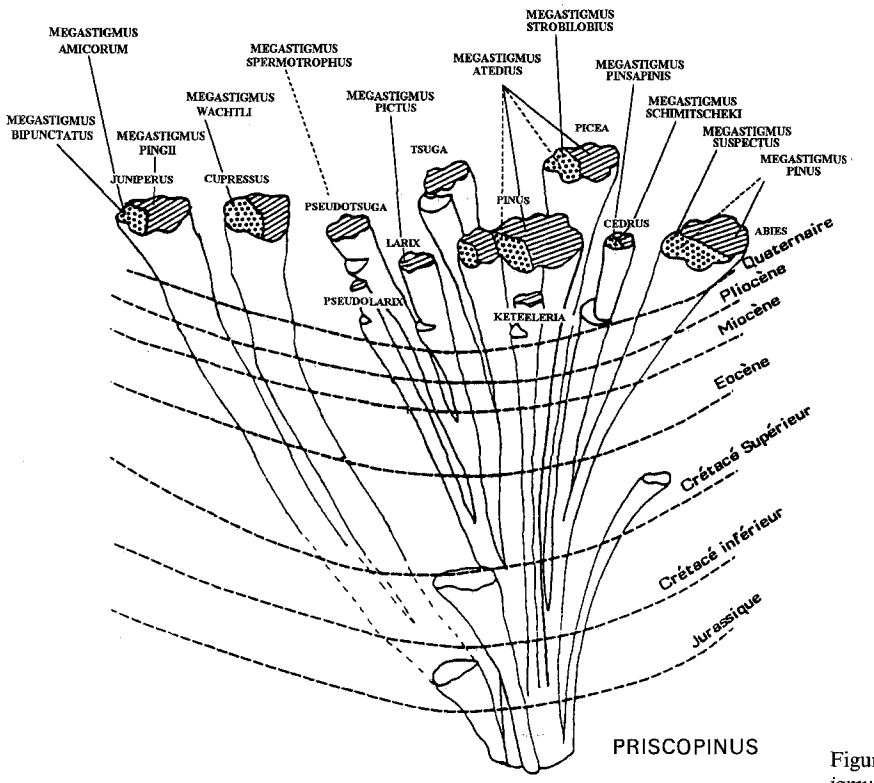


Figure 2. Distance Wagner tree, based on Rogers' distance, of populations of *Megastigmus*. The cophenetic correlation was 0.866 and the percent standard deviation of Fitch and Margoliash was 40.352.

tity observed between the populations of *M. spermotrophus* tended to indicate that this species has only undergone a limited amount of differentiation since its introduction into Europe. The genetic identity might be assumed to proceed from a common origin in north America.

The branching pattern of the UPGMA dendrogram (fig. 1) also revealed that most of the populations of *M. suspectus* infesting *Abies* spp. probably constitute a monophyletic group. All of these populations were collected in the same location. Thus, the differentiation observed in the *Abies* group seems to be resource-related rather than to be caused by geographic isolation. The genetic identity values calculated between the populations infesting *A. numidica*, *A. pardei*, and *A. pinsapo* ranged 0.98 to 1.00. Such values were generally found when comparing conspecific populations rather than distinct species[48]. By contrast, the lowest genetic identity value observed between the populations of *M. suspectus* from *A. nordmanniana* and the populations of *M. suspectus* infesting the other *Abies* species was 0.77, i.e. a value included between the average values reported for sibling and non sibling insect species[49]. A greater number of electrophoretic loci and a larger sample are required to clarify the relationships between these populations, and oviposition experiments could also be useful. The populations presently identified as *M. suspectus* may therefore be interpreted in several ways: a group of species, a group of sibling species, or conspecific populations in a speciation process adaptive to each *Abies* species. In any event, the populations infesting *A. nordmanniana* probably belong to a species other than *M. suspectus*. Also interesting was the case of another specialist of fir seeds, *M. pinus* from *A. nobilis*, that clustered with *M. suspectus* (from Europe) although it was introduced from north America.

The *Abies* seed chalcids and the species infesting *Cedrus atlantica* seemed very close. The last species was considered to be a variety of *M. suspectus* for a long time, and was also recorded on some *Abies* species[15, 20, 25]. Pintureau et al.[25] did not find any genetic difference between the two species when they performed a limited electrophoretic analysis, but these authors used morphological and biological characters to differentiate them. *M. schimitscheki* from *Cedrus libani* seemed more genetically-distant from the *Abies*-infesting group, but the low number of individuals that were analyzed prevented any definitive conclusion. By contrast, the rather large genetic distance observed between *M. atedius* (*Picea orientalis*) and the populations of *M.*



▩ Area in Europe, Asia Minor and Mediterranean Region.
▧ Area in the rest of the World.

Figure 3. Specificity of some *Megastigmus* species, compared to the phylogeny of the Conifers. (After Roques[21], modified).

suspectus infesting *A. alba* was unexpected (fig. 1). This introduced seed chalcid is presently considered the only *Megastigmus* species to attack two different conifer genera, *Pinus* and *Picea*, but only a limited population of *Picea orientalis* could be analyzed at that time, and the results should also be considered as provisional. Therefore, it would be very interesting to compare populations developing on *P. orientalis* with those infesting *Pinus strobus* to ascertain their specific status.

The seed chalcid species infesting Cupressaceae clustered together. The genetic identity value was 0.92 between two populations of *M. amicorum* with different juniper hosts, and corresponded more to the value typical of conspecific populations than to those of distinct species. This result may be due to the limited size of the sample analyzed, and should be carefully considered. However, closely related species may also show high genetic identity values[48]. Onthe other hand, the genetic data tended to separate the species infesting the Mediterranean Cupressaceae from these infesting alpine (and Chinese) junipers. These differences may result from a divergent evolution of the *Megastigmus* populations in the two biogeographic regions following the Quaternary glaciations[21]. Moreover, the genetic identity between the populations of *M. amicorum* infesting two Mediterranean Junipers, *Juniperus phoenicea* and *J.oxycedrus*, was similar to that observed between each of these two populations and the cypress seed chalcid, *M. wachtli*. We may therefore speculate that *Megastigmus* populations could easily colonize different *Juniperus* species.

The colonization of Angiosperm seeds was first considered as a recent evolution of the seed chalcid group[21]. The genetic distances observed between *M. rosae* and both the other species infesting *Rosa* spp. and that infesting *Pistacia* suggested a more complex process, that may have involved several pathways.

The Torymidae family is presumed to have appeared at the beginning of the Tertiary[50], i.e. quite concurrently with the process of diversification in conifers. Figure 3 presents a tentative representation of the relationships between the evolution of conifers and the present host range of *Megastigmus* seed chalcids in Europe. A distinct seed chalcid species appeared to be related to each of the surviving secondary branches that successively separated from the mutual Gymnosperm trunk. Our enzymatic data fitted this representation, mainly when the Wagner tree was considered. The early separation of Cupressaceae and Pinaceae may underly the large genetic distance observed between the respective seed chalcid species, whereas the closeness of the populations infesting *Abies* and *Cedrus* may result from the late separation of the two conifer genera. A further genetic analysis of natural populations of the European spruce

seed chalcid, *M. strobilobius* Ratz., would also be informative on account of the genus *Picea* in the course of conifer evolution (fig. 3). More generally, it has been assumed that the present seed chalcid species evolved from a common ancestor that colonized the seeds of the early conifers, and then diversified through adaptive radiation[21]. This could explain the worlwide distribution of the genus *Megastigmus* and the genetic proximity of species attacking the same conifer genus in the Old World and in the New World, e.g. *M. suspectus* and *M. pinus*. By contrast, such correlations between host and insect phylogenies have not been observed by Roininen et al.[9], where *Euura atra* did not appear to be congruent with the phylogeny of willow species. In many other cases, when the phylogenetics of host plants and insects have been compared, no correlation has been found (e.g. Futuyma and McCafferty[45]).

Specificity of *Megastigmus* seed chalcids has thus tended to remain at a generic level although limited genetic differentiation with respect to host species was already noticeable in some populations. Seed chalcids are still capable of colonizing newly introduced tree species that are phylogenetically close to their usual host[21]. However, some limits may exist. For example, Annila[17] showed that colonization of exotic *Abies* spp. by *M. specularis* in Finland was dependent on the length of the female ovipositor relative to cone size and thickness. Other factors could result in reproductive isolation of seed chalcids. Strong selection for efficient host location and oviposition behaviour guided by plant-specific chemical stimuli can lead to specialization on suitable host species which grows locally. All of the *Megastigmus* species have a single generation per year but large differences were shown at the time of adult emergence with respect to host tree, e.g. in *Abies* spp.[30], as well as in the duration of prolonged diapause[13, 21]. By contrast, the haplodiploid genetic system directly exposes unfavorable genes to selection either in the male (arrhenotokous species) or in the female (thelytokous species). Unfertilized females thus produce male (or female) progeny, which would tend to increase the frequency of a gene favouring a new feeding behaviour[51]. Coupled with the short duration of a chalcid generation, this feature would favour a rapid adaptation to a new host.

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