Experimental hybridisation between *Aphis grossulariae* and *Aphis triglochinis* (Sternorrhyncha: Aphididae)

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Abstract. *Aphis triglochinis* and *A. grossulariae* clones from southern Poland produced fertile hybrid eggs under experimental conditions. Established hybrid clones expressed normal parthenogenetic reproduction but bisexual generations were obtained only in three hybrid clones out of twenty six. Fertile F_1 hybrid eggs were obtained in one hybrid clone. Morphological and host-specificity features of *A. grossulariae* dominated in the majority of hybrid clones. The present results do not exclude the possibility of natural hybridisation of studied aphid species. Natural hybrids may be difficult to detect because of their "pure" morphological and host-specificity features.

INTRODUCTION

The possibility of hybridising and producing viable and fertile progeny is an important feature of biparental species, being emphasized by the reproductive species concepts, including the biological species concept (e.g. Mayr, 1982; Dobzhansky, 1970; Paterson, 1993). Aphids are mostly biparental species, and reproductive isolation is important feature of aphid species (Shaposhnikov, 1987; Blackman, 1995; Rakauskas, 1998a). Hybridisation studies might supply important information on the taxonomic status of the forms involved in a complex (Müller, 1985; Shaposhnikov, 1987; Guldemond, 1990a; etc.), although aphid crossing experiments are rather complicated (Hales et al., 1997).

Species of the genus *Aphis* L. inhabiting currants in Europe [*Aphis grossulariae* Kaltenbach, 1843, *A. triglochinis* Theobald, 1926, and *A. schneideri* (Börner, 1940)] present certain taxonomic problems (Hille Ris Lambers & Dicker, 1965; Stroyan, 1984; Rakauskas, 1998b). Detailed biosystematic studies of this species complex have therefore been undertaken. It appeared that the three species are rather distinct in their life-cycles and host specificity, although they share the same winter hosts. *A. schneideri* is monoecious holocyclic on *Ribes* spp., *A. grossulariae* is holocyclic facultatively heteroecious between *Ribes* spp. and Onagraceae herbs, and *A. triglochinis* is holocyclic obligatorily heteroecious between *Ribes* spp. and Brassicaceae, Boraginaceae and Asteraceae herbs (Rakauskas, 1993). Sibling species may occur on the summer hosts of *A. triglochinis* (Rakauskas, 1998b). Many of the morphological characters exploited in the keys to discriminate between the three species appeared to be unreliable. Nevertheless, morphometric analysis of numerous specimens of all morphs revealed morphological features, ensuring separation of all morphs of *A. grossulariae*, *A. triglochinis* and *A. schneideri* (Rakauskas, 1998c). All three species have the same chromosome number (2n = 8), and preliminary karyotype analysis suggests that *A. triglochinis* is more closely

related to *A. schneideri* than to *A. grossulariae*, but this is inconsistent with morphological and host specificity data (Turčinavičienė et al., 1997). DNA-s of the three species appeared to be different when analysed by means of the randomly amplified polymorphic DNA polymerase chain reaction technique. Four out of 13 primers applied produced bands that were polymorphic among the three species. Based on the numbers of bands shared in common, *A. grossulariae* seems to be more closely related to *A. schneideri* when compared with *A. triglochinis* (Turčinavičienė et al., 1999). Thus, morphological, life-cycle, host specificity and DNA analysis data suggest that *A. grossulariae*, *A. triglochinis* and *A. schneideri* are good, well-defined species. Nevertheless, with regards to the similarity of karyotype and controversial references on the bionomics of the three species (see Rakauskas, 1998b, c), experimental interspecific hybridisation studies have been undertaken. Data on *A. grossulariae* × *A. schneideri* and *A. schneideri* × *A. triglochinis* crossing results are already published (Rakauskas, 1999a, b).

The aim of this work was to study the possibility of hybridisation between *A. grossulariae* and *A. triglochinis* under experimental conditions.

MATERIAL AND METHODS

Five clones of *A. grossulariae* and three clones of *A. triglochinis* originating from southern Poland were used for interspecific crossing experiments in Katowice (southern Poland) in 1987, each clone starting from a single fundatrix or fundatrigenia (Table 1). Hybridisation experiments were the continuation of morphological, life-cycle and host specificity studies of the two species (Rakauskas, 1993). This ensured the precise documentation of the morphology and bionomics of parental clones and provided data for obtaining key morphological characters and canonical discrimination functions to distinguish between various morphs of *A. grossulariae* and *A. triglochinis* (see below). Sixteen *A. grossulariae* ♀ × *A. triglochinis* ♂ and ten reciprocal crossings were tried. Ten oviparae and one male of the alternative species were isolated in muslin branch-tip cages on currant bushes for each cross. The construction of cages ensured the isolation of 10 cm of the terminal part of the

TABLE 1. *A. grossulariae* and *A. triglochinis* clones used for the crossing experiments in Katowice (Poland) in 1987 (c. v. – cultivated variety).

Sampling locality	Sampling date	Host plant	Subsequent clone No.
		A. grossulariae	
Katowice	May 12	red currant c. v.	B1
Katowice	May 12	*Ribes aureum* Pursh.	E1
Katowice	May 12	*Ribes aureum* Pursh.	E2
Katowice	May 12	*Ribes aureum* Pursh.	F1
Katowice	May 23	gooseberry c. v.	F2
		A. triglochinis	
Zabrzeg, Katowice distr.	May 18	black currant c. v.	A1
Zabrzeg, Katowice distr.	May 18	black currant c. v.	A2
Katowice	July 14	*Rorippa silvestris* (L.) Bess.	A3

currant shoot (see Rakauskas & Rupais, 1983). Groups of 5 to 10 gynoparae of the same clone were used to receive newly born oviparae larvae. Gynoparae were obtained from cages of clones of each respective species and their morph was confirmed under a stereoscopic microscope (16×) before releasing them into branch-tip cages on currants. After depositing progeny gynoparae were removed and fixed in alcohol for subsequent morphological analysis, as were males. This ensured that the oviparae were virgin. It is noteworthy that *A. grossulariae* and *A. triglochinis* have no sexuparae: those remigrating from summer host plants are gynoparae producing only larvae of future oviparae. Currant shoots were isolated early in autumn, at the moment when the first gynoparae appeared, and males were not yet present. This (and careful examination of the shoot by means of 2.5× magnifying glass) eliminated the possibility of any wild eggs inside the cage. Eggs of these species have never been found in Katowice at the beginning of September.

Hybrid eggs were obtained from thirteen crosses (Table 2). Intraspecific interclonal and intraclonal crosses were also performed. Eggs were subsequently transferred (together with fragments of shoots on which they were deposited) to Vilnius (Lithuania) and maintained in field conditions throughout the winter. This was performed by attaching the fragments of the shoots containing hybrid eggs to the appropriate tip shoots of the field grown black currant bushes (mid-ripening variety "Derliai") inside muslin branch-tip cages. Currant shoots used were carefully checked using 2.5× magnifying glass, to confirm

TABLE 2. *A. triglochinis* and *A. grossulariae* successive crossings, with information on the amount of live (black shining) eggs obtained, hatching and maturation success of fundatrices and subsequent hybrid clone designation.

Maternal and paternal clones		No. of eggs	Hatching success (%)	Fx maturation success (%)	Subsequent hybrid clone
females	males				
		1. *A. grossulariae* ♀ × *A. triglochinis* ♂			
B1	A3	23	69.6	100	gt1–2
B1	A1	21	47.6	100	gt3–4
E1	A1	24	20.8	100	gt5–6
E1	A3	9	33.3	100	gt7–8
E2	A3	32	53.1	100	gt9–10
E2	A1	16	56.3	100	gt11–12
E2	A2	59	35.6	100	gt13–14
F2	A2	68	45.6	100	gt15–16
F2	A3	20	60.0	100	gt17–20
		2. *A. triglochinis* ♀ × *A. grossulariae* ♂			
A1	F2	21	42.9	100	tg1–3
A1	E1	11	45.5	100	tg4–6, 9–10
A1	E2	7	14.3	100	tg7
A1	B1	21	14.3	100	tg8

the absence of any naturally-occurring aphid eggs. The surface of the second-year currant shoot of the exploited variety is smooth, without any crannies for hidden eggs. Hatched larvae were able to crawl to bursting buds and continue their development. Hatching larvae were counted daily, as were mature fundatrices. Single fundatrices were isolated separately in branch tip muslin cages afterwards, initiating hybrid clones for subsequent morphological analysis and host specificity tests. Thus twenty six hybrid clones were started and propagated throughout 1988 in Vilnius. Rearing methods were the same as described earlier (Rakauskas, 1993). The list of clones is presented in Tables 3 and 4: clone number indicates also the origin of the hybrid clone: gt1 means the first hybrid clone from the crossing scheme *A. grossulariae* ♀ × *A. triglochinis* ♂, tg1 – the first hybrid clone from the reciprocal crossing.

The fundatrix and twenty specimens (when available) of the main morphs (alatae and apterae from winter and summer hosts) of each hybrid clone were mounted in Faure-Berlese fluid on microscope slides for morphological analysis. Two methods were used for the morphological identification of hybrid clones. First, the identification was attempted using common key characters (Rakauskas, 1998c). Numbers of additional hairs on the ultimate rostral segment (for fundatrices, apterous viviparous females from currants and summer host plants, alate viviparous females from currrants, gynoparae, oviparae and males) and numbers of secondary rhinaria on the third antennal segment (for alate viviparous females from summer hosts) were used as key characters. Second, canonical variates analysis, a method that has proved very useful in distinguishing closely related aphid species (e.g., Blackman, 1992; etc.), was applied. Morphometric data of pure *A. schneideri* and *A. grossulariae* clones (see above) were used for calculating the canonical discrimination functions (CDF) for each morph. Variables used in the CDF were selected on the basis of their discriminatory power: those having the smallest partial Wilks' Lambda were taken when calculating the CDF for every morph (for details see StatSoft, 1995, Chapter 2). List of variables used for calculating the CDF for every morph is presented in Table 5. Wider information on the aphid material used has been already published (Rakauskas, 1998c). The obtained CDF values were subsequently counted for every hybrid specimen of every morph, and standard box and whisker plot procedure was applied for morphological determination of various morphs of every hybrid clone. Examples illustrating the morphological identification procedure of the alate currant-inhabiting viviparous females are presented in Fig. 1 (using the key characters) and Fig. 2 (exploiting the CDF). Every morph of each hybrid clone was treated as having the morphology of a particular species if the range of the studied character or CDF values in that morph was covered by the range of the same character of that particular species. Thus, alate viviparous females (currant morph) of the hybrid clones gt6–11,

gt15–18 were determined as having the morphology of *A.grossulariae* both by means of key character (Fig. 1) and CDF values (Fig. 2). The hybrid clone morph was treated as morphologically tending towards a particular species if the 25–75% box area of the studied character or CDF value of that morph was overlapped by the range of that particular species. For example, alate viviparous females (currant morphs) of hybrid clones gt3–5, gt12–14 were determined by means of key character as tending morphologically towards *A. grossulariae* (Fig. 1). The hybrid morph was treated as morphologically intermediate if the 25–75% box area of the studied character or CDF value for that morph was between the ranges of both species.

TABLE 3. Morphological and biological features of the experimental hybrid clones (*A. grossulariae* ♀ × *A. triglochinis* ♂) showing the summer host specificity (+ , normal propagation on respective hosts; ± , poor propagation; − , no propagation), morphological pecularities of different morphs of each clone (fx – fundatrix; apt, al – apterae and alatae from currants; aptII, alII – apterae and alatae from summer hosts; gyn – gynoparae; male – males; ovip – oviparae; t, → t – morphology as in *A. triglochinis* or tending to it; g, → g – morphology as in *A. grossulariae* or tending to it; i – intermediate morphological features; n – morph not obtained; 0 – morph obtained, but not measured) when performing identification by common key characters (key) or by means of canonical discrimination function (CDF, see in material and methods), and the overall morphology of the clone (summary). Figures in morph column – No. of analysed specimens of respective morph.

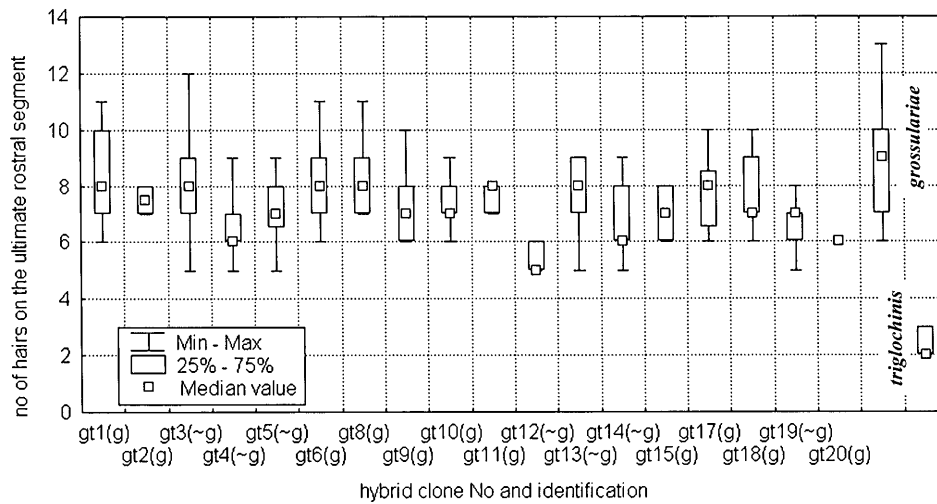| Clone No.	Acceptance of summer hosts of respective species		Morphology			
	grossulariae	*triglochinis*	Morph/No.	Key	CDF	Summary
gt1	±	−	fx/1	g	→ g	→ g
			apt/1	g		
			al/7	g	→ g	
			aptII/0	0	0	
			alII/n	n	n	
gt2	+	−	fx/1	g	→ g	g
			apt/4	g	g	
			al/2	g	g	
			aptII/8	g	g	
			alII/2	g	g	
gt3	+	−	fx/1	g	→ g	→ g
			apt/3	→ g	→ g	
			al/19	→ g	g	
			aptII/0	0	0	
			alII/n	n	n	
gt4	+	−	fx/1	g	g	→ g
			apt/13	→ g	→ g	
			al/10	→ g	g	
			aptII/18	→ g	→ g	
			alII/n	n	n	
gt5	+	−	fx/0	0	0	g
			apt/12	g	g	
			al/12	→ g	g	
			aptII/12	g	g	
			alII/18	g	→ g	
			gyn/2	g	g	
			ovip/5	g	g	
gt6	+	−	fx/1	g	→ g	g
			apt/10	g	g	
			al/19	g	g	
			aptII/8	→ g	g	
			alII/23	g	→ g	
gt8	±	−	fx/1	g	→ g	g
			apt/15	g	g	
			al/9	g	g	
			aptII/0	0	0	
			alII/n	n	n	
gt9	+	−	fx/1	g	g	g
			apt/11	g	g	
			al/23	g	g	
			aptII/0	0	0	
			alII/n	n	n	
gt10	+	−	fx/1	g	g	g
			apt/5	g	g	
			al/17	g	g	
			aptII/11	→ g	→ g	
			alII/13	g	→ g	
gt11	+	−	fx/0	0	0	g
			apt/10	→ g	g	
			al/4	g	g	
			aptII/13	g	g	
			alII/21	g	g	
gt12	killed by predators	killed by predators	fx/1	g	→ g	g
			apt/4	g	g	
			al/3	→ g	g	
			aptII/n	n	n	
			alII/n	n	n	
gt13	±	−	fx/1	g	g	g
			apt/7	g	g	
			al/12	→ g	→ g	
			aptII/n	n	n	
			alII/n	n	n	
gt14	+	−	fx/1	g	g	g
			apt/10	g	→ g	
			al/18	→ g	g	
			aptII/10	→ g	g	
			alII/19	g	→ g	
gt15	+	−	fx/1	g	g	g
			apt/5	g	g	
			al/5	g	g	
			aptII/9	→ g	→ g	
			alII/n	n	n	
gt16	+	−	fx/1	g	g	g
			apt/0	0	0	
			al/0	0	0	
			aptII/15	g	g	
			alII/9	g	g	
gt17	+	−	fx/1	g	0	g
			apt/9	→ g	g	
			al/16	g	g	
			aptII/0	0	0	
			alII/0	0	0	
			gyn/2	g	g	
			male/9	g	→ g	
			ovip/1	g	i	
gt18	+	−	fx/1	g	g	g
			apt/1	g	g	
			al/19	g	g	
			aptII/2	g	g	
			alII/n	n	n	
gt19	±	−	fx/1	g	g	g
			apt/10	→ g	g	
			al/11	→ g	g	
			aptII/0	0	0	
			alII/0	n	n	
gt20	−	−	fx/1	g	g	g
			apt/6	g	→ g	
			al/1	g	g	
			aptII/n	n	n	
			alII/n	n	n	

Fig. 1. Box and whisker plot of the key morphological character for the alate viviparous females of *A. grossulariae* and *A. triglochinis* and hybrid clones *grossulariae* × *triglochinis* (currant morphs).

Graphical data for other morphs of all hybrid clones (similar to those presented in Figs 1–2 for alate viviparous females) are available from the author on request. Morphological identification of fundatrices was different, because only one fundatrix of every clone was available. Scatterplot analysis procedure was performed in this case, an example being presented in Fig. 3. In total, five morphs of the majority of hybrid clones were evaluated morphologically by means of key characters and CDF. Thus, ten evaluations (key characters and CDF for each morph) of every hybrid clone were obtained. Every hybrid clone was afterwards summarized as having certain overall morphological features on the basis of these ten evaluations. For example, hybrid clone gt1 (Table 3) had 2 evaluations (when using CDF) as tending morphologically to *A. grossulariae*, and 3 evaluations (when applying key characters) as being morphologically identical with *A. grossulariae*. In overall determination, this hybrid clone was treated as tending morphologically to *A. grossulariae*, since CDF performs identification on the basis of more characters. Following the same procedure, hybrid clone gt2 was determined as morphologically identical with *A. grossulariae*, clones gt3–4 as tending morphologically to *A. grossulariae*, and so on (Tables 3–4). Discussion of morphological characters elsewhere in this paper concerns this overall morphological determination of the clone, unless otherwise stated.

All calculations were done using the STATSOFT statistical package STATISTICA for WINDOWS 5.1 (StatSoft, 1995).

Host specificity and life cycle analysis of every hybrid clone were performed in the same way as described earlier (Rakauskas, 1993). Potted *Epilobium adenocaulon* Hausskn., *Chamaenerion angustifolium* (L.) Scop. (Onagraceae, summer hosts of *A. grossulariae*), *Cardamine amara* L. (Brassicaceae) and *Myosotis palustris* L. (Boraginaceae; both summer hosts of *A. triglochinis*) plants were tested as potential summer hosts for every clone. Transfers of alate females were repeated (if first transfers were unsuccessful) at weekly intervals until this morph was no longer available. Groups of five migrants were used for each transfer test. This was one of the reasons for insufficient numbers of certain morphs used for the morphological analysis in some clones (e.g. lack of alate viviparae from currants in clone gt16). When only a few winged viviparae were obtained, they all were used for transfer experiments. Hybrid clones that produced sexuales were crossed both intra- and inter clonally (Table 6). Five oviparae and one male were used in each F_1 crossing variant. Backcrossing with pure *A. triglochinis* and *A. grossulariae* clones was not performed because of the lack of sexuales in pure clones of these species in autumn 1988 in Vilnius.
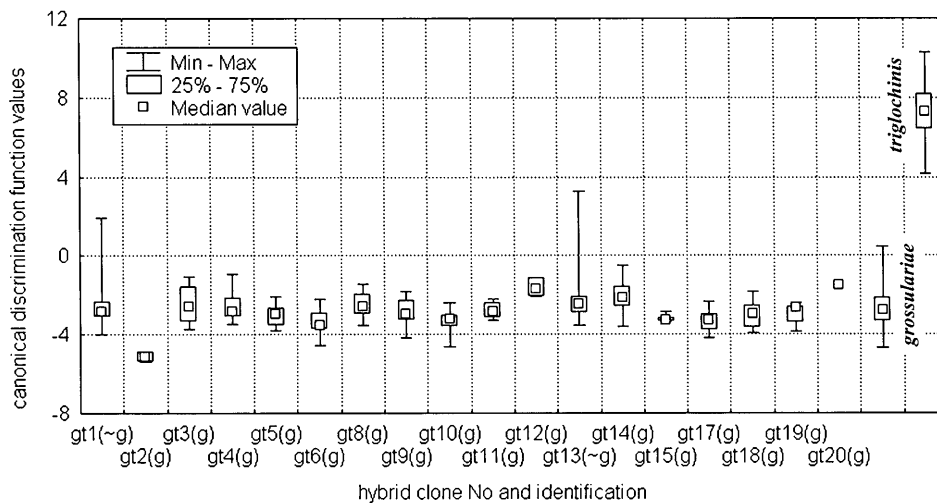

Fig. 2. Box and whisker plot of canonical discrimination function values for the alate viviparous females of *A. grossulariae* and *A. triglochinis* and hybrid clones *grossulariae* × *triglochinis* (currant morphs).

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Table 4. Morphological and biological features of the experimental hybrid clones (*A. triglochinis* ♀ × *A. grossulariae* ♂). Abbreviations as in Table 3.

Clone No.	Acceptance of summer hosts of respective species		Morphology			
	grossulariae	triglochinis	Morph/No.	Key	CDF	Summary
tg1	–	±	fx/1	t	t	t
			apt/n	n	n	
			al/6	t	t	
			aptII/n	n	n	
			alIII/n	n	n	
tg4	+	–	fx/1	g	g	g
			apt/2	g	g	
			al/1	g	g	
			aptII/0	0	0	
			alIII/n	n	n	
tg5	±	–	fx/1	g	g	g
			apt/2	g	g	
			al/2	g	g	
			aptII/0	0	0	
			alIII/n	n	n	
tg6	?	?	fx/1	g	g	g
			apt/n	n	n	
			al/n	n	n	
			aptII/n	n	n	
			alIII/n	n	n	
tg7	+	–	fx/1	g	g	g
			apt/5	g	g	
			al/4	g	g	
			aptII/4	g	g	
			alIII/1	g	g	
			gyn/31	g	g	
			male/16	g	g	
			ovip/6	g	g	
tg8	±	–	fx/1	g	→g	g
			apt/9	g	g	
			al/16	→g	g	
			aptII/0	0	0	
			alIII/n	n	n	
tg9	+	–	fx/0	0	0	g
			apt/3	g	g	
			al/10	g	g	
			aptII/8	g	g	
			alIII/0	0	0	

RESULTS

It seems obvious that *A. triglochinis* and *A. grossulariae* are capable of hybridising under experimental conditions: 26 hybrid clones were obtained, and they reproduced normally by means of parthenogenesis (Tables 3–4). On the other hand, the morphological and host-specificity features of the hybrid clones are puzzling. Information on secondary host specificity and the morphological features of hybrid clones can be summarized as follows (overall morphological determination above the line, host specificity below the line; →gross., →trigl. – overall morphology or host specificity tending to the respective species; monoec.? – hybrid clone probably monoecious on currants, rejected all proposed secondary hosts; ? – lack of information).

A. grossulariae ♂ × *A. triglochinis* ♀ crosses:

$$11\frac{gross.}{gross.} : 3\frac{gross.}{\rightarrow gross.} : 1\frac{\rightarrow gross.}{\rightarrow gross.} : 2\frac{\rightarrow gross}{gross.} : 1\frac{gross.}{?} : 1\frac{gross.}{monoec.?}$$

In *A. triglochinis* ♂ × *A. grossulariae* ♀ crosses:

$$3\frac{gross.}{gross.} : 2\frac{gross.}{\rightarrow gross.} : 1\frac{gross.}{?} : 1\frac{trigl.}{\rightarrow trigl.}$$

An interesting result is that none of the 26 hybrid clones possessed intermediate morphology. 22 clones were morphologically identical with *A. grossulariae*, 3 clones tended morphologically to this species, 1 clone was identical with *A. triglochinis*. *A. grossulariae* morphology was dominant whether *A. grossulariae* or *A. triglochinis* oviparous females were used for the crossing experiments.

A confusing phenomenon appeared in hybrid clones gt3, gt5, gt10 and gt14: certain alate viviparous females (currant morphs) had some of the antennal hairs finely acute and relatively long (Fig. 4d). This is one of the key characters of the third currant-inhabiting species, *Aphis schneideri* (Fig. 4b). The existence of *A. grossulariae* specimens having *A. schneideri*-like antennal hairs has been already documented for pure clones of *A. grossulariae* (Rakauskas, 1998c).

None of the hybrid clones had the pure host specificity of *A. triglochinis*. Even hybrid clone tg1, being morphologically indistinguishable from *A. triglochinis*, developed poorly on *A. triglochinis* summer hosts. Alate viviparous females of this clone accepted *Cardamine amara* and *Myosotis palustris* as summer hosts, fed and deposited progeny, although no progeny reached adulthood on these plants. Alate viviparous females of this clone rejected *Epilobium adenocaulon* and *Chamaenerion angustifolium* as summer hosts. Fourteen hybrid clones normally accepted *Epilobium adenocaulon* and *Chamaenerion angustifolium* as summer hosts, eight hybrid clones propagated on these plants more poorly.

One hybrid clone (gt20, having *A. grossulariae* morphology) did not accept any of the tested herbaceous plants as summer hosts, and also failed to finish its entire life-cycle on currants. In hybrid clone gt5 (morphologically *A. grossulariae*), some gynoparae and oviparae appeared on currants, but no sexuales were produced on summer hosts. This is in accordance with the previous data on facultative heteroecy and monoecy in *A. grossulariae* (Gusynina, 1963; Savzdarg & Ponomareva, 1978).

Three hybrid clones succeeded in producing a bisexual generation. In *A. grossulariae* ♀ × *A. triglochinis* ♂ crossings, hybrid clone gt5 produced gynoparae and subsequently oviparae on currants. Hybrid clone gt17 gynoparae and males were produced on *Epilobium adenocaulon*, and oviparae (after transfer of gynoparae to winter host) on currants. In reciprocal crosses, hybrid clone tg7 produced plenty of gynoparae and males on *Epilobium adenocaulon*, and subsequently oviparae on currants. Morphologically, gynoparae and males of all three hybrid clones were similar to *A. grossulariae* (Figs 5–6). Hybrid gt5 and tg7 oviparae were morphologically *A. grossulariae*, whereas the only analysed hybrid gt17 ovipara was morphologically intermediate (Fig. 7). It is remarkable that numbers of scent plaques on the hind tibiae of hybrid oviparae were markedly reduced when compared with both parental species: in twelve analyzed hybrid oviparae, the numbers of scent plagues on the hind tibia were from 3 to 78 (mean value 33.36). The respective figures for *A. grossulariae* are 43–116 (85.00) and

Clone No (identification)

◆ tg1 (triglochinis)
▲ tg4 (grossulariae)
● tg5 (grossulariae)
■ tg6 (grossulariae)
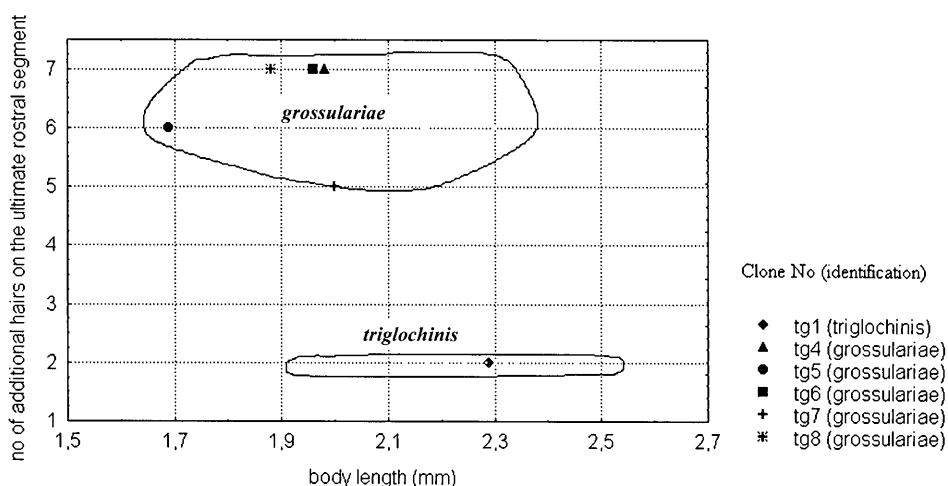+ tg7 (grossulariae)
✳ tg8 (grossulariae)

Fig. 3. Scatterplot of the individual main key character values plotted against the body length of hybrid fundatrices (*A. triglochinis* × *A. grossulariae* crossings) showing the distribution of the same values in *A. grossulariae* and *A. triglochinis* fundatrices.

for *A. triglochinis* 56–83 (72.20). It is noteworthy that the absence of scent plagues on the hind tibiae of oviparae is characteristic of *A. schneideri* (Rakauskas, 1998c). The malformation of scent plagues in hybrid oviparae might explain the low success of F_1 crossing (Table 6). Intra-clonal crosses of hybrid clones resulted in the appearance of winter eggs only in hybrid clones gt5 and tg7, but nothing hatched from these eggs the following spring. F_1 crosses with the *A. schneideri*-like hybrid clone st1 (*A. schneideri* × *A. triglochinis* crosses, Rakauskas, 1999b) resulted in eggs, except for the case when hybrid clone gt17 oviparae were used. Successful overwintering and hatching of these eggs occurred only in the st1 × tg7 cross. Unfortunately, all five F_1 cross fundatrices were killed by an *Anthocoris* predatory bug (as the cage muslin was damaged) previous to their maturation. The F_1 cross results suggest the possibility that hybrid *A. grossulariae* × *A. triglochinis* clones possessing normal potential for bisexual reproduction may be found in the field.

DISCUSSION AND CONCLUSIONS

A. triglochinis and *A. grossulariae* clones from southern Poland are able to produce fertile hybrid eggs under experimental conditions. Established hybrid clones expressed normal parthenogenetic reproduction, and the bisexual generation appeared in three hybrid clones. Nevertheless, F_1 crosses inside the hybrid clones were not successful. Pure clones of *A. triglochinis* and *A. grossulariae* also demonstrated reduced possibilities for bisexual reproduction in 1988 in Vilnius (for details, see Rakauskas, 1999a). Therefore, it remains unclear whether hybrid *A. grossulariae* × *A. triglochinis* clones had reduced potential for bisexual reproduction and hybrid breakdown is a possible postzygotic isolating mechanism between these species. The fact that the crossing of the hybrid *A. triglochinis* × *A. grossulariae* clone tg7 male with the hybrid *A. schneideri* × *A. triglochinis* clone st1 oviparae resulted in fertile egg production supports the hypothesis of natural hybridisation between the studied species. Nevertheless, the present results are rather preliminary. Successful experimental hybridisation does not necessarily mean the possibility of natural hybridisation. Experimental interspecific crossings have been successfully performed in aphid genera *Dysaphis* (Shaposhnikov, 1987, etc.), *Myzus* (Müller, 1969), *Cryptomyzus* (Guldemond, 1990a, b, etc.), *Ovatus* (Müller & Hubert-Dahl,

TABLE 5. Morphological characters exploited for calculating canonical discrimination functions used for the discrimination of the respective morphs of *A. triglochinis* and *A. grossulariae*. Abbreviations as in Table 4.

| Characters	Morphs							
	fx	apt	al	aptII	alII	gyn	male	ovip
Siphunculus length				+				
Antennal segm. III length		+					+	+
Longest hair on ant. segm. III length			+				+	
Antennal segm. IV length	+					+		+
Antennal segm. V length					+			
Basal length of ant. segm. V(VI)				+		+		
Processus terminalis length	+	+	+	+	+			+
Articular width of ant. segm. III	+							
Maximum width of ant. segm. III			+					
No. of secondary rhinaria on ant. segm. III			+		+	+		
No. of secondary rhinaria on ant. segm. IV						+		
Ultimate rostral segment length	+	+		+			+	
No. of hairs on ultimate rostral segment		+						
Length of cauda								+
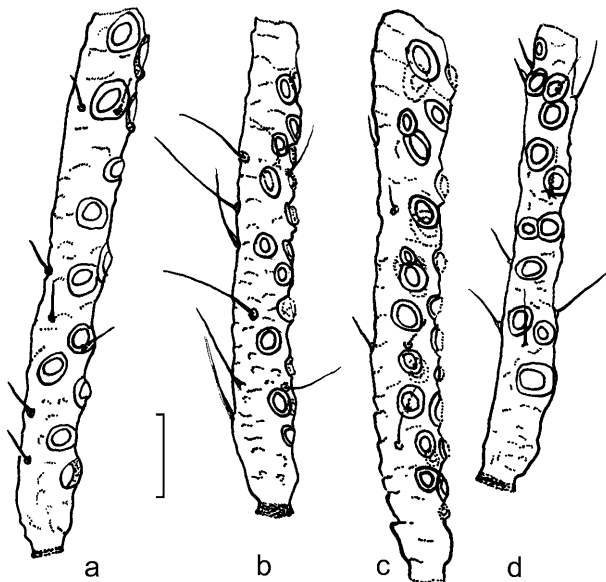
Fig. 4. Third antennal segment of alate viviparous females (currant morphs) of *A. grossulariae* (a), *A. schneideri* (b), *A. triglochinis* (c) and hybrid (*A. grossulariae* ♀ × *A. triglochinis* ♂) clone gt10 (d). a – Vilnius, 26.vi.1984, cultivated red currant; b – Vilnius, 10.v.1983, cultivated red currant; c – Vilnius, 26.v.1988, cultivated black currant; d – Vilnius, 20.vi.1988, cultivated black currant. Scale: 0.05 mm.

TABLE 6. Hybrid *A.triglochinis* x *A. grossulariae* F_1 crossing scheme, with information on the amount of live (black shining) eggs obtained and hatching success.

Maternal and paternal clones		No. of eggs	Hatching success (%)
females	males		
1. intraclonal crossings			
gt5	gt5	3	0
gt17	gt17	0	
tg7	tg7	8	0
2. interclonal crossings			
gt5	st1 (*A. schneideri* × *A. grossulariae*)	4	0
gt17	st1 (*A. schneideri* × *A. grossulariae*)	0	
tg7	st1 (*A. schneideri* × *A. grossulariae*)	1	0
st1	gt17	2	0
st1	tg7	9	55.6

1979), and the *Aphis fabae* Scopoli complex (Müller, 1982; Thieme, 1988). Natural prezygotic isolating mechanisms might be rather sophisticated and fragile (Guldemond et al., 1994; Guldemond & Dixon, 1994; Thieme & Dixon, 1996), and be easily circumvented by the experimental procedure. So, information on successful experimental hybridisation reinforces the need for the study of natural isolating mechanisms between the species involved, such as sex pheromone specificity, the circadian rhythms of sex pheromone release and male activity, and other aspects of possible species-specific mate recognition systems. Crossing experiments need to be repeated with clones from other parts of the species distribution area, because the possibilities to produce hybrids may

differ in different populations (Hewitt, 1990). DNA analysis of parental and hybrid clones (e.g. microsatellites and mitochondrial DNA techniques, see Hales et al., 1997; Sunnucks et. al., 1997) would help to confirm the identity of the crosses and indicate the degree of introgression between natural populations of this species group. The question of natural hybridisation between *A. triglochinis* and *A. grossulariae* is important not only in a taxonomic context, but also in terms of the practical needs of currant pest management. The appearance of hybrid specimens having certain morphological characters of *A. schneideri* (Fig. 4d), the third European species of the currant-inhabiting complex of the genus *Aphis*, is of special interest. Because of hybridisation, clones with the morphological features of *A. grossulariae* might appear, but being monoecious (as hybrid clone gt20) or facultatively monoecious (as hybrid clone gt5) on currants. This might explain previous data on monoecy in *A. grossulariae* (Gusynina, 1963; Savzdarg & Ponomareva, 1978). We have recently found *A. triglochinis* clones in Finland that seem to be facultatively heteroecious (Rakauskas, Turčinavičienė, unpubl.), a characteristic of *A. grossulariae*.

The present data raise certain questions concerning the genetic control of morphological characters. Despite the
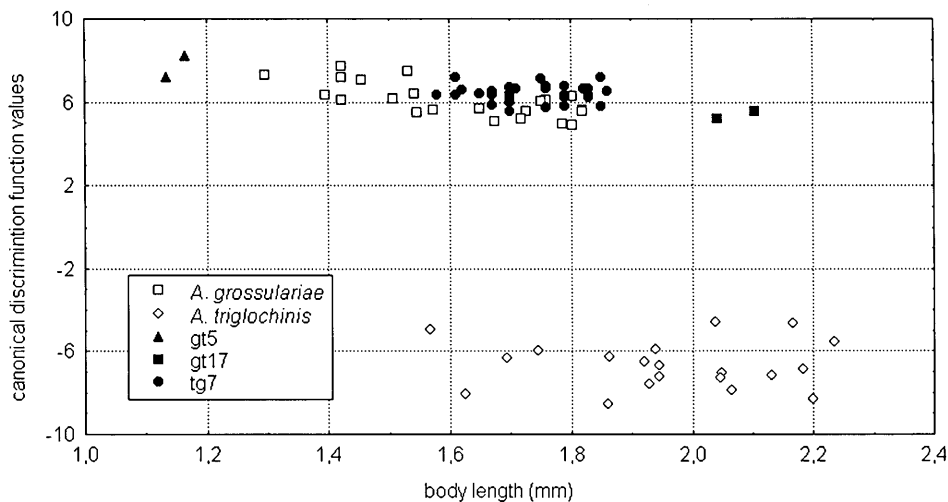

Fig. 5. Scatterplot of the individual canonical discrimination function values plotted against the body length of *A. grossulariae*, *A. triglochinis* and hybrid gynoparae.
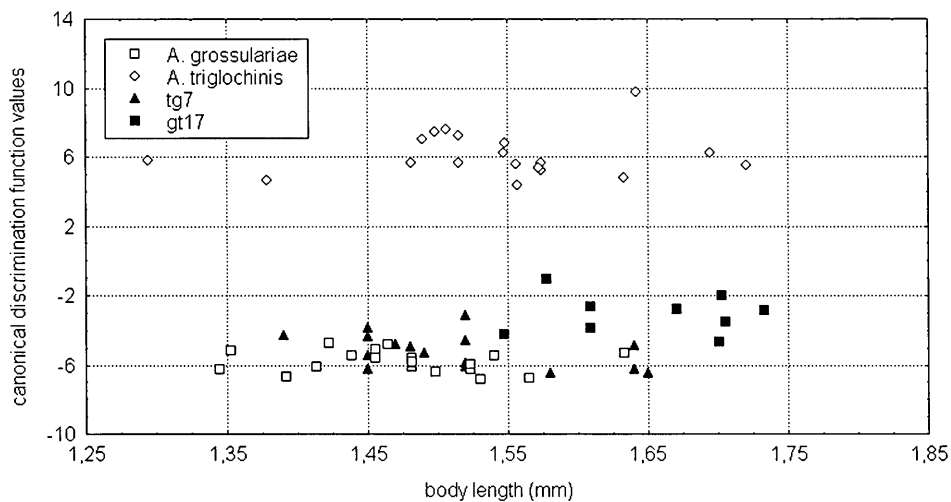
Fig. 6. Scatterplot of the individual canonical discrimination function values plotted against the body length of *A. grossulariae*, *A. triglochinis* and hybrid males.

14 characters used for calculating the CDF for the different morphs, all hybrid clones were identical with (23 clones of 26) or clearly tended to (3 clones) one of the species. The absence of morphologically intermediate clones is consistent with a hypothesis of the monogenic control of morphological characters, with *A. grossulariae* characters being dominant. That does not conform to the common understanding of the genetic control of developmental processes (Blackman, 1999, pers. comm.), although it is not absolutely impossible, e.g., the "polyphene" mutation in *Drosophila* affects various morphological characters, such as eyes, thorax, tarsi and wings, but it is known to be a single gene mutation (Severtsov, 1987: 24). Single gene pleiotropic effects on general morphology has been also reported in plants (Bohmert et al., 1998). The phenomenon, whereby all hybrid clones are identical with maternal species, can be explained as due to action of maternal genes (Lewin, 1996: 1141–1179), or fertilization errors, such as gynogenesis (Ham & Veomett, 1980: 605), also due to hybridogenesis (Cherfas, 1981). Nevertheless, most of our hybrid clones

that were obtained when using *A. triglochinis* oviparae (6 out of 7) were also identical with *A. grossulariae*.

The majority of hybrid clones, 22 of 26 tested, had the host specificity of *A. grossulariae*: they developed on *Epilobium adenocaulon* and *Chamaenerion angustifolium*, and rejected *Cardamine amara* and *Myosotis palustris*. The scarcity of hybrid clones having intermediate host preferences between *A. grossulariae* and *A. triglochinis* suggests monogenic control of this character. Monogenic control of host specificity has already been reported for the raspberry aphid *Amphorophora rubi* (Kaltenbach) (Briggs, 1965). Guldemond (1990a) also suggested that host plant specificity in the aphid genus *Cryptomyzus* might be controlled by only a few genes.

It may be that natural crosses between *A. grossulariae* and *A. triglochinis* (if they exist in the field) are hardly detectable, because of their similarity to one of the parental species. The dominance of morphological and host specificity features of *A. grossulariae* is in accordance with the information on the rarity of *A. triglochinis*: it is rather uncommon on currants, at least in Europe (Hille Ris Lambers & Dicker, 1965; Cichocka, 1980;
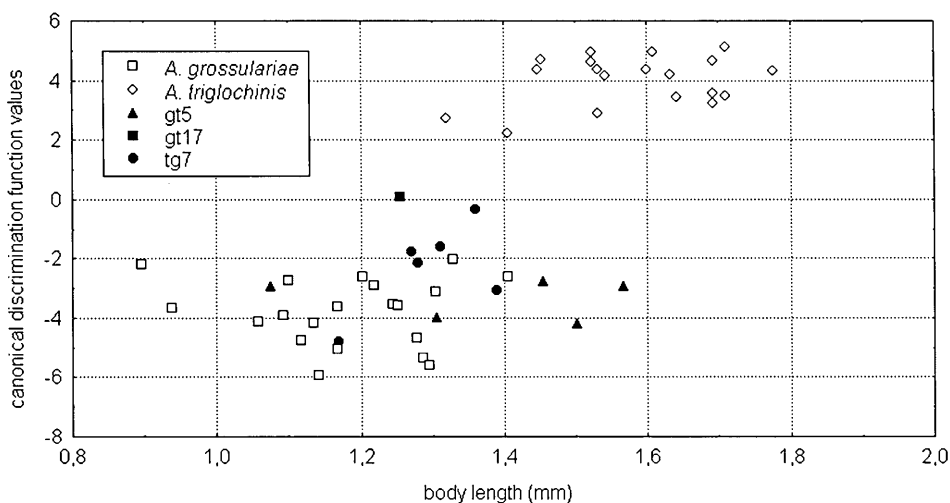

Fig. 7. Scatterplot of the individual canonical discrimination function values plotted against the body length of *A. grossulariae*, *A. triglochinis* and hybrid oviparae.

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Holman & Pintera, 1981). *A. triglochinis* may be rare, if its phenotype is just a recessive homozygote of the gene, whose dominant allele causes *A. grossulariae* features. Morphology of *A. schneideri*, *A. grossulariae* and *A. triglochinis* suggests that the latter species should be more phylogenetically distant from the first two (Stroyan, 1984; Rakauskas, 1998c). Remaudière (1993) even places them in separate subgenera. According to this, *A. grossulariae* × *A. schneideri* crosses should be more successful than *A. grossulariae* × *A. triglochinis*, but it seems not to be true (Rakauskas, 1999a). On the other hand, the more distantly related are the parental species, the higher is probability that hybrids will look like one or the other of them. The phenomenon has been explained in other systems by preferential gene expression of the maternal allele (Wu et al., 1997), maternal gynogenesis (Makeeva, 1989), or elimination of paternal chromosomes during distant hybridogenesis (Cherfas, 1981). The explanation in the present case is not yet known.

REFERENCES

BLACKMAN R.L. 1992: The use of ordination techniques to discriminate within pest aphid species complexes. In Sorensen J.T. & Footit R.G. (eds): *Ordination in the Study of Morphology, Evolution and Systematics of Insects*. Elsevier, Amsterdam, pp. 261–275.

BLACKMAN R.L. 1995: Species concepts and realities. *Bull. Entomol. Res.* **85**: 1–4.

BOHMERT K., CAMUS I., BELLINI C., BOUCHEZ D., CABOCHE M. & BENNING C. 1998: AGO1 defines a novel locus of Arabidopsis controlling leaf development. *EMBO Journal* **17**: 170–180.

BRIGGS J.B. 1965: The distribution, abundance, and genetic relationships of four strains of the Rubus aphid [(Amphorophora rubi (Kalt.)] in relation to raspberry breeding. *J. Hort. Sci.* **40**: 109–117.

CHERFAS N.B. 1981: Natural gynogenesis and hybridogenesis. In Kirpichnikov V.S. (ed): *Genetic Bases of Fish Selection*. Springer, Berlin-Heidelberg, pp. 255–266.

CICHOCKA E. 1980: *Mszyce roslin sadowniczych Polski [Orchard Aphids of Poland]*. State Scientific Publishers, Warszawa, 119 pp. (in Polish).

DOBZHANSKY T. 1970: *Genetics of the Evolutionary Process*. Columbia University Press, New York, 505 pp.

GULDEMOND J.A. 1990a: Evolutionary genetics of the aphid Cryptomyzus, with a preliminary analysis of the inheritance of host plant preference, reproductive performance and host-alteration. *Entomol. Exp. Appl.* **57**: 65–76.

GULDEMOND J.A. 1990b: *On Aphids, their Host Plants and Speciation (A Biosystematic Study of the Genus Cryptomyzus)*. Ponsen & Looijen, Wageningen, 159 pp.

GULDEMOND J.A. & DIXON A.F.G. 1994: Specificity and daily cycle of release of sex pheromones in aphids: a case of reinforcement? *Biol. J. Linn. Soc.* **52**: 287–303.

GULDEMOND J.A., TIGGES W.T. & DE VRIJER P.W.F. 1994: Circadian rhythm of sex pheromone production and male activity of coexisting sibling species of Cryptomyzus aphids (Homoptera: Aphididae). *Eur. J. Entomol.* **91**: 85–89.

GUSYNINA L.M. 1963: [Aphids of the genus Aphis L. on currants and gooseberry.] *Proc. Biol. Inst. Siberian Div. Acad. Sci. USSR* **10**: 77–81 (in Russian).

HALES D.F., TOMIUK J., WÖHRMANN K. & SUNNUCKS P. 1997: Evolutionary and genetic aspects of aphid biology: A review. *Eur. J. Entomol.* **94**: 1–55.

HAM R.G. & VEOMETT M.J. 1980: *Mechanisms of Development*. C.V. Mosley, St. Louis-Toronto-London, 843 pp.

HEWITT G.M. 1990: Divergence and speciation as viewed from an insect hybrid zone. *Can. J. Zool.* **68**: 1701–1715.

HILLE RIS LAMBERS D. & DICKER G.H.L. 1965: Aphis triglochinis Theobald, 1926, as a pest of red currant and black currant (Homoptera, Aphididae). *Entomol. Ber.* **25**: 5–6.

HOLMAN J. & PINTERA A. 1981: Übersicht der Blattlause (Homoptera, Aphidoidea) der Rumanischen Sozialistischen Republik. *Studie ČSAV 15*. Academia, Prague, 128 pp.

LEWIN B. 1996: *Genes V*. Oxford University Press, Oxford-New York-Tokyo, 1272 pp.

MAKEEVA A.P. 1989: [Results of distant hybridisation of species from different families of Cypriniformes]. In Kirpichnikov V.S. (ed.): *[Genetics in Aquaculture. Proceedings of 3th All-Union Conference of Genetics, Selection and Hybridization of Fishes]*. Nauka, Leningrad, pp. 153–168 (in Russian).

MAYR E. 1982: *The Growth of Biological Thought: Diversity, Evolution, and Inheritance*. Harvard University Press, Cambridge, MS, 974 pp.

MÜLLER F.P. 1969: Bastardierungsversuche zur Feststellung von Isolierungsmechanismen zwischen nahe verwandten Formen in der Gattung Myzus Passerini (Homoptera: Aphididae). *Biol. Zbl.* **88**: 147–164.

MÜLLER F.P. 1982: Das problem Aphis fabae. *Z. Angew. Entomol.* **94**: 432–446.

MÜLLER F.P. 1985: Biotype Formation and Sympatric Speciation in Aphids (Homoptera: Aphidinea). *Entomol. Gen.* **10**: 161–181.

MÜLLER F.P. & HUBERT-DAHL M.L. 1979: Wirtwechsel, Generationenfolge und reproduktive Isolation von Ovatus crataegarius (Walker, 1850) und O. insitus (Walker, 1849). *Dt. Entomol. Z.* **26**: 241–253.

PATERSON H. 1993: *Evolution and the Recognition Concept of Species: Collected Writings*. Harvard University Press, Cambridge, MA, 234 pp.

RAKAUSKAS R. 1993: On the biology of the Ribes-infesting Aphis species in Central Europe. In Kindlman P. & Dixon A.F.G. (eds): *Critical Issues in Aphid Biology. Proceedings of the 4th International Symposium on Aphids*. Institute of Entomology, České Budějovice, pp. 113–122.

RAKAUSKAS R. 1998a: What is the (aphid) species? In Nieto Nafria J.M. & Dixon A.F.G. (eds): *Aphids in Natural and Managed Ecosystems*. Universidad de León, León, pp. 447–451.

RAKAUSKAS R. 1998b: On the identity of Aphis triglochinis Theob. In Cichocka E., Goszczynski W. & Wiech K. (eds): *Aphids and Other Homopterous Insects 6*. Polish Academy of Science, Warszawa, pp. 5–16.

RAKAUSKAS R. 1998c: Morphometric analysis of European species of the genus Aphis (Sternorrhyncha: Aphididae) inhabiting Ribes. *Eur. J. Entomol.* **95**: 239–250.

Rakauskas R. 1999a: Hybridisation between Aphis grossulariae and Aphis schneideri (Sternorrhyncha: Aphididae): An experimental approach. *Eur. J. Entomol.* **96**: 401–408.

Rakauskas R. 1999b: Experimental hybridisation of the currant inhabiting Palaearctic species of the genus Aphis L. (Hemiptera: Aphididae). *Acta Zool. Lituan.* **9(3)**: 96–106.

Rakauskas R. & Rupais A. 1983: Biology of the green apple aphid in Lithuania. *Acta Entomol. Lituan.* **6**: 20–30.

Remaudière G. 1993: Pucerons nouveaux et peu connus du Mexique (12e Note) Aphis (Bursaphis) solitaria (McVicar Baker) (Homoptera, Aphididae). *Rev. Fr. Entomol. (N.S.)* **15**: 97–106.

Savzdarg E.E. & Ponomareva M.S. 1978: [Diagnosis of aphid species on currants and their dynamics in central districts of RSFSR.] *Proc. Timirjazev Agric. Acad.* **1**: 150–158 (in Russian).

Severtsov A.S. 1987: *Fundamentals of the Theory of Evolution.* Moscow University Press, Moscow, 320 pp. (in Russian).

Shaposhnikov G.Kh. 1987: Organisation (structure) of populations and species, and speciation. In Minks A.K. & Harrewijn P. (eds): *Aphids. Their Biology, Natural Enemies and Control. Vol. 2A.* Elsevier, Amsterdam, pp. 415–430.

StatSoft, Inc. 1995: *STATISTICA for Windows (Computer program manual).* Tulsa, OK.

Stroyan H.L.G. 1984: Aphids – Pterocommatinae and Aphidinae (Aphidini). *Handbooks for the Identification of British Insects. Vol. II, Part 6.* Royal Entomological Society, London, 232 pp.

Sunnucks P., De Barro P.J, Lushai G., Maclean N. & Hales D. 1997: Genetic structure of an aphid studied using microsatellites: cyclic parthenogenesis, differentiated lineages and host specialization. *Mol. Ecol.* **6**: 1059–1073.

Thieme T. 1988: Zur Biologie von Aphis fabae mordwilkowi Borner und Janisch, 1922 (Hom., Aphididae). *J. Appl. Entomol.* **105**: 510–515.

Thieme T. & Dixon A.F.G. 1996: Mate recognition in the Aphis fabae complex: daily rhythm of release and specificity of sex pheromones. *Entomol. Exp. Appl.* **79**: 85–89.

Turčinavičienė J., Rakauskas R. & Lazutka J. 1997: Karyotypes of three aphid species: Aphis triglochinis, Aphis grossulariae and Aphis schneideri. *Biologija (Vilnius)* **4**: 60–62.

Turčinavičienė J., Sužiedėlis K. & Rakauskas R. 1999: DNA analysis of the currant inhabiting aphid species of the genus Aphis L. by means of the randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR). *Acta Zool. Lituan.* **9(1)**: 178–182.

Wu Qingjiang, Fu Hongtuo & Ye Yuzhen 1997: Effect of enzymic gene dosage on the distant hybridisation of fishes. *Acta Hydrobiol. Sin.* **21**: 143–151.