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HOST-BASED MORPHOMETRIC DIFFERENTIATION IN THREE *CINARA* SPECIES (INSECTA: HEMIPTERA: APHIDIDAE) FEEDING ON *PINUS EDULIS* AND *P. MONOPHYLLA*

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ABSTRACT.—*Cinara edulis* (Wilson), *C. terminalis* (Gillette and Palmer), and *C. wahtolca* Hottes were all larger when feeding on *Pinus monophylla* Torr. & Frem. than when feeding on *P. edulis* Engelm. Almost all nonsetal morphometric characters were longer in aphids on the former of these pinyon pines. Although mouthpart characters also followed this pattern of size in *C. edulis* and *C. wahtolca*, rostrum length showed the opposite pattern in *C. terminalis* and was shorter when on *P. monophylla*. This reversal in size pattern suggests that mouthpart size can be independent of overall aphid size. Principal components analyses corroborate the univariate statistics and we discuss the contribution of various characters to the principal components. We compare environmental induction and environmental selection as explanations for the observed size differences and discuss the taxonomic implications of our results.

Key words: Aphididae, Cinara, *morphometrics, insect,* Pinus edulis, Pinus monophylla.

Host-based races of phytophagous insects have become commonly reported in the entomological literature, evidence coming from allozyme (Berlocher 1999), DNA sequence (Brown et al. 1996), morphological (Carson et al. 1982, Pappers and Ouborg 2002), and ecological (Eubanks et al. 2003) data. Aphids (Hemiptera: Aphididae), all species of which are plant phloem feeders, also form host-adapted races (Via 1999, Shufran et al. 2000). Aphid species can exhibit morphological differentiation along host plant lines (Blackman 1981), and indeed, the morphological divergence can be high enough to prompt taxonomists to name new species (Blackman 1987).

We wished to see if morphological adaptation to closely related host plants was evident in *Cinara* (Aphididae: Lachninae), a group of aphids that feed exclusively on conifers in the Cupressaceae and Pinaceae (Eastop 1972) and that can be of economic importance (Kfir et al. 1985, Watson et al. 1999, Penteado et al. 2000). Although pinyon pines are occasional economic resources (Lanner 1981), the *Cinara* that feed on the principal pinyon species in the U.S., *Pinus edulis* Engelm. and *P. monophylla* Torr. & Frem., very rarely have any economic impact (see Palmer 1926 for the only known record of injurious pinyon *Cinara*) and are not subject to the high selective pressures common to aphids in agricultural systems. Pinyons are small- to medium-sized pines whose ranges extend over the mountainous terrain of Mexico and the southwestern U.S. *Pinus edulis* grows at elevations between 1600 m and 2300 m in Arizona, Utah, New Mexico, Colorado, southwestern Texas, far western Oklahoma, and in an isolated population in southern California. *Pinus monophylla* grows at elevations between 1300 m and 2200 m in southern and eastern California, Nevada, southwestern Utah, and western Arizona. These 2 species of pinyon (and hence their concomitant *Cinara*) are largely allopatric, with a few parapatric areas that also contain hybrids (Trombulak and Cody 1980, Gafney and Lanner 1987). Because of their limited elevational range, pinyon distribution is scattered and island-like, especially in the Great Basin (Critchfield and Little 1966). Pinyons can form monocultural woodlands but are more often found in large stands of pinyon-juniper woodlands, oak-pinyon scrub, or mixed stands of other pines, usually *P. ponderosa* Dougl.

Fourteen species of *Cinara* are recorded from *P. edulis*, and a subset of 4 from *P. monophylla* (Voegtlin and Bridges 1988). Favret and Voegtlin (2004) have shown enough genetic divergence between *C. wahtolca* Hottes feeding on *P. edulis* and *P. monophylla* to posit the possibility of separate, host-affiliated species.

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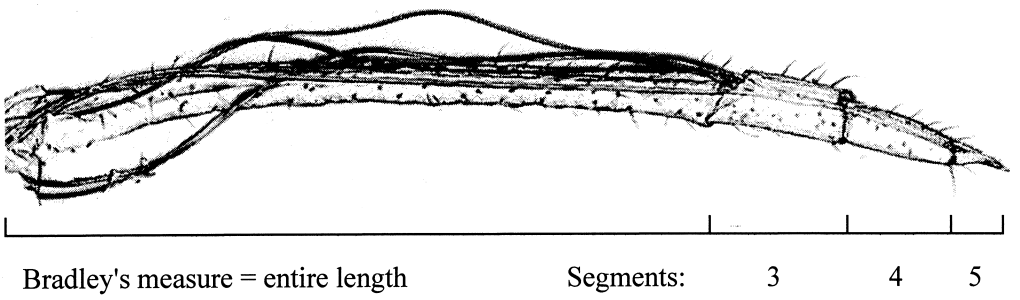

Fig. 1. Rostrum of *C. edulis*.

Here we sought supporting evidence of speciation by examining morphological divergence between populations of *C. wahtolca* on the 2 hosts.

Two other species of pinyon-feeding *Cinara* were collected in large numbers. *Cinara edulis* (Wilson) and *C. terminalis* (Gillette and Palmer), like *C. wahtolca*, were collected across the full range of the pinyons. Even if *C. wahtolca* represented only a single species, host-based morphological differences may be present if induced environmentally. We therefore analyzed the other 2 *Cinara* species to see if they might follow the same host-based trend as *C. wahtolca.*

The Rostrum of *Cinara*

The morphology of the rostrum is widely used in aphid taxonomy. The length of the whole rostrum and some of its segments, the shape and number of accessory setae on the 4th rostral segment, and the shape of the 4th and 5th segments combined are used in many identification keys (Richards 1972, Corpuz-Raros and Cook 1974, Eastop 1979, 1987, Cook 1984). Furthermore, the structure of the feeding apparatus is often correlated with host plant. For instance, many grass-feeding aphids have short, blunt rostra, whereas aphids that feed on the bark of woody hosts have long rostra (Heie 1980). *Cinara* fall into the latter category, and their long, lance-shaped rostrum is a synapomorphy for the genus (Heie 1988).

The rostrum of *Cinara* is composed of 5 segments. The first 2 are longer and unpigmented, the next 2 are shorter and sclerotized, and the terminal segment is usually considerably reduced (Fig. 1). The number of setae on the 4th segment is conserved within a species and is taxonomically informative (Eastop 1972, Pepper and Tissot 1973). Overall length of the rostrum in *Cinara* is also taxonomically informative (Voegtlin 1976) and is correlated with the part of the woody host the aphid feeds on: the thicker the bark, the longer the rostrum. In other words, root- and trunk-feeding aphids have longer rostra than branch-feeding aphids, which in turn have longer rostra than shoot-feeding aphids (Bradley 1961). This correlation is not without exceptions, and some species are occasionally found at atypical feeding sites. For instance, we have found *C. ponderosae* (Williams), normally a shoot feeder, on roots, and *C. puerca* Hottes, normally a root feeder, on shoots.

As there is a close affinity between rostrum morphology and host identity, we predicted that any morphological differences between *Cinara* species feeding on *P. edulis* and *P. monophylla* would be most pronounced in the rostrum. We predicted that host-based differences in the morphology of the rostrum would be relatively independent of any host-based differences in the morphology of other parts of the aphid. To test this hypothesis, we compared the rostrum of aphids from the 2 pinyon host species. We also performed principal components analyses with a suite of other morphological characters.

MATERIALS AND METHODS

Collections and Measurements

Collections of 123 colonies of viviparous (live-bearing) *C. edulis*, *C. terminalis*, and *C. wahtolca* were made on *P. edulis* and *P. monophylla* across the hosts' full ranges during the

summers of 1997–2001. To ensure that we were comparing appropriate specimens, we did not analyze material from pinyon hybrids. Material was collected by visual searching and by beating sheet. Specimens were placed into 70% ethanol and later cleared and mounted to slides in Canada balsam. Identifications were made with Hottes's (1960) and Blackman and Eastop's (1994) keys and with reference to the types. All specimens were deposited in the Illinois Natural History Survey insect collection.

We analyzed approximately 3 aphids from each collected colony. A total of 80 viviparous alatae (winged aphids) and 134 viviparous apterae (wingless aphids) of *C. edulis* were collected and measured. Fifty-seven and 92 viviparous apterae of *C. terminalis* and *C. wahtolca,* respectively, were collected and measured. Insufficient numbers of alatae of these 2 latter species were collected, so they were omitted from the analyses. Characters that were obscured or otherwise difficult to measure were omitted on some individuals; therefore the actual sample sizes vary for each character (Table 1).

Characters

We examined the aphids' rostra to determine if their morphology was correlated with host identity. The 2nd rostral segment in *Cinara* telescopes into the 1st (Hottes 1954), making it difficult to obtain a precise measure of overall length. The sclerotized portion of the stylet groove has provided a useful proxy for rostrum length (Bradley 1961, Foottit and Mackauer 1990). Measurements of this feature, hereafter referred to as Bradley's measure, were made using an ocular micrometer on a compound microscope. Rostra that were bent beyond ~30° or whose basal portion was obscured by the aphid's body or head were omitted.

We counted accessory setae on the 4th rostral segment, which include all setae on the segment except the 6 on the distal end. Measurements of the lengths of the 3rd and 4th rostral segments were made with a camera lucida and Zidas digitizing pad. It is difficult to obtain consistently accurate measurements of the diminutive 5th segment, so it was not included in the analyses.

In addition to the rostral characters, a suite of other characters was chosen based on successful use in previous studies (Bradley 1961, Eastop 1972, Foottit and Mackauer 1990, Foottit 1992), ease of measurement, and low level of distortion under the pressure of a cover slip (Foottit 1992). Some standard characters such as counts of setae on the genital plate or siphuncular cones were omitted because they were hard to examine accurately in a large enough sample of specimens, or because preliminary analysis indicted that they were largely uninformative. Measurements were made with a camera lucida and digitizing pad, including the overall body length; lengths of the 3rd, 4th, and 5th antennal segments; lengths of the femur, tibia, and ventral aspects of the 2 tarsal segments; and lengths of the longest seta on the midpoint of the 3rd antennal segment, dorsal side of the hind tibia, and 5th abdominal tergum. We counted the number of setae on the basal portion of the 6th antennal segment and the 8th abdominal tergite.

Analysis of Variance and Principal Components Analysis

We performed analyses of variance (ANOVA) for all 4 rostral characters, and for the ratio of Bradley's measure to body length and the ratio of 3rd and 4th rostral segments. Ratios were used to control for correlation between length of the rostral segment and overall size of the aphid. Analyses of variance compared characters of apterae of all 3 species and alatae of *C. edulis* feeding on *P. edulis* and *P. monophylla.*

To determine if nonrostral characters were subject to the same level of host-affiliated specialization as the rostrum, we performed univariate statistical analyses, ANOVAs, on them and on several ratios as well. The 3 ratios were composed of closely related characters, such that they likely would be subject to similar selective pressure and correlated response (Price and Langen 1992). Ratios were of the 2 metatarsal segments, the metafemur and the metatibia, and the 4th and 5th antennal segments.

We also performed multivariate analyses, in the form of principal components analysis (PCA; Seal 1968, Pimentel 1979, Jackson 1991), using all characters except the composite characters (ratios) and Bradley's measure, which was omitted to increase sample size. In PCA the correlation between all characters is assessed in a correlation matrix, and the contribution of each measure to overall morphometry (shape and size) of the individual aphid is summarized. PCA not only compares the contribution of

various measures to the principal components, but it also compares individual aphids with each other along each of the principal component axes. In this way morphometrically distinct groups can be recognized (Jeffers 1967, Pimentel 1979). SYSTAT 10 software (SPSS, Inc., Chicago, IL) was used to perform the PCA. We used Quartimax rotation, a standard means of rotating the data points in n-dimensional space to optimize their explanatory power, keeping those principal components that explained 1% or more of the morphometric variation. Lastly, we plotted PCA scores for individual aphids on 2-dimensional graphs and performed ANOVAs with the principal components to compare host-affiliated groups statistically (Pimentel 1979).

RESULTS

Univariate Analyses of Variance

Differences in univariate rostral morphology were found across all 4 samples (*C. edulis* alatae and apterae of all 3 species) and for all 6 characters and ratios (first 6 lines of Table 1). All 3 length measures were greater for *P. monophylla*–feeding *C. edulis* alatae than they were for those aphids on *P. edulis.* The same pattern was found for *C. wahtolca* apterae as well as for rostral segments 3 and 4 in *C. edulis* apterae. The rostral measurements for *C. terminalis* apterae showed the reverse distinction, however, and all 3 characters were shorter for *P. monophylla*–feeding aphids than they were for those aphids feeding on *P. edulis.* Both alatae and apterae of *C. edulis* had more setae on their 4th rostral segment if they fed on *P. edulis,* but *C. wahtolca* had more setae when feeding on *P. monophylla.* The ratio of Bradley's measure to body length was different for all samples except *C. wahtolca* apterae, and the ratio of rostral segments 3 and 4 was different for *C. terminalis* and *C. wahtolca* apterae (Table 1).

Differences in nonrostral characters were also evident (lines 7 to 21 of Table 1). Where differences were seen, in all 4 samples, measurements of nonsetal body parts were longer on *P. monophylla* than on *P. edulis*. The metatibia, 3rd and 5th antennal segments, and 2nd metatarsal segment were longer in all 4 sets of *P. monophylla*–feeding aphids. All 3 setal measurements were longer in *P. monophylla*–feeding *C. wahtolca* than in those feeding on *P. edulis,* but of the other 9 combinations (3 setal measurements for *C. edulis* alatae, *C. edulis* apterae, and *C. terminalis*), a difference was seen in only 1: *C. edulis* had longer dorsal abdominal setae when feeding on *P. edulis.* Of the setal counts the only observed differences were more setae on the 8th abdominal tergum in *P. edulis*–feeding *C. edulis* alatae than in those feeding on *P. monophylla,* and the reverse scenario for *C. wahtolca* apterae. No differences were observed in the count of setae on the base of the 6th antennal segment or for the ratio of metatibia to metafemur. Differences were observed in the ratio of 4th to 5th antennal segments in all samples except *C. terminalis,* and in the ratio of 2nd to first metatarsal segments for *C. terminalis* and *C. wahtolca* (Table 1).

Principal Components Analyses and Multivariate ANOVAs

The average 1st principal component score for individual aphids was different for all 4 sets of aphids, as determined by ANOVA; means of the 1st component score for individual aphids were negative for those feeding on *C. edulis* and positive for those feeding on *P. monophylla* (Table 2). There was no significant difference in the means of the 2nd, 4th, or 5th principal component scores between *P. edulis*– and *P. monophylla*–feeding *Cinara.* Differences in the 3rd principal component were seen in *C. edulis* and *C. terminalis* apterae (Table 2). Figure 2 plots these 2 groups of aphids on axes representing the 1st and 3rd components.

The direction and magnitude of the contribution of each character to PCA showed a pronounced role for the antennal and tarsal segments, tibia, and body length in the 1st principal component (Fig. 3). The rostral segments contributed to the 1st principal component in *C. edulis* alatae and *C. edulis* and *C. wahtolca* apterae, but negatively to the 3rd principal component in *C. terminalis* apterae. Likewise, the femur contributed greatly to the 1st principal component in the apterae of all 3 species, but the femur and the tibia contributed more to the 3rd principal component in the *C. edulis* alatae, the tibia being in a negative direction. The lengths of setae tended to contribute positively to the 2nd principal component, although the length of the setae on tergum 5 in *C. edulis* apterae contributed most to the 3rd component.

Setal counts in both morphs of *C. edulis* contributed negatively to the 1st component

TABLE 1. Morphological measurements (mm). Sample size, mean, range, and level of significance in ANOVA: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Species and morph	*C. edulis* alatae						
Host plant	*P. edulis*			*P. monophylla*			
Measurements	*N*	Mean	Range	*N*	Mean	Range	Sig.
Bradley's measure	26	1.82	1.66–2.08	26	1.90	1.73–2.03	**
Bradley's measure / Body	25	0.541	0.430–0.880	25	0.497	0.412–0.675	*
Rostrum 3	41	0.232	0.195–0.262	39	0.241	0.222–0.263	**
Rostrum 4	41	0.188	0.160–0.213	39	0.196	0.177–0.214	***
Rostrum 3 / Rostrum 4	38	1.24	1.13–1.39	38	1.23	1.11–1.37	
Count of setae on rostrum 4	41	16.0	12–22	38	14.2	11–16	***
Length of seta on antennal segment 3	39	0.048	0.027–0.062	39	0.047	0.027–0.076	
Length of seta on hind tibia	41	0.065	0.028–0.101	39	0.059	0.036–0.083	
Length of seta on 5th abdominal tergum	36	0.035	0.008–0.063	36	0.028	0.008–0.063	
Hind tibia	40	2.59	1.78–3.08	39	3.06	2.48–3.48	***
Hind femur	41	1.55	1.20–3.20	39	1.62	1.34–1.87	
Tibia / Femur	40	1.78	1.68–2.02	39	1.89	1.68–2.03	
3rd antennal segment	39	0.480	0.395–0.559	39	0.534	0.447–0.549	***
4th antennal segment	39	0.231	0.170–0.291	39	0.267	0.222–0.333	***
5th antennal segment	39	0.260	0.215–0.313	39	0.297	0.219–.0315	***
Antennal segment 4 / segment 5	39	0.887	0.705–1.02	39	0.901	0.746–1.15	
1st tarsal segment	40	0.121	0.100–0.134	39	0.128	0.095–0.142	***
2nd tarsal segment	40	0.263	0.228–0.301	37	0.283	0.261–0.312	***
Tarsal segment 2 / segment 1	40	2.19	2.01–2.46	37	2.21	1.99–2.70	
Count of setae on base of antennal segment 6	38	11.3	8–16	36	10.8	6–14	
Count of setae on 8th abdominal tergum	40	14.0	10–19	37	12.4	10–16	***

Species and morph	*C. edulis* apterae						
Host plant	*P. edulis*			*P. monophylla*			
Measurements	*N*	Mean	Range	*N*	Mean	Range	Sig.
Bradley's measure	48	1.82	1.53–2.14	46	1.87	1.58–2.19	
Bradley's measure / Body	48	0.555	0.444–0.736	46	0.497	0.393–0.608	***
Rostrum 3	59	0.238	0.203–0.275	73	0.243	0.206–0.277	*
Rostrum 4	61	0.195	0.168–0.219	73	0.201	0.174–0.232	**
Rostrum 3 / Rostrum 4	59	1.22	1.08–1.44	73	1.21	1.06–1.38	
Count of setae on rostrum 4	60	15.5	11–20	73	14.8	12–19	*
Length of seta on antennal segment 3	60	0.049	0.022–0.078	73	0.052	0.033–0.077	
Length of seta on hind tibia	59	0.060	0.023–0.092	67	0.062	0.030–0.089	
Length of seta on 5th abdominal tergum	58	0.023	0.007–0.053	71	0.013	0.007–0.055	***
Hind tibia	59	2.56	1.98–3.11	66	2.84	2.11–3.65	***
Hind femur	59	1.42	1.09–2.60	69	1.56	1.24–1.85	***
Tibia / Femur	60	1.84	1.60–2.02	66	1.81	1.61–1.99	
3rd antennal segment	60	0.467	0.305–0.610	73	0.516	0.385–0.609	***
4th antennal segment	59	0.214	0.138–0.280	73	0.248	0.171–0.331	***
5th antennal segment	59	0.248	0.179–0.322	73	0.278	0.183–0.343	***
Antennal segment 4 / segment 5	59	0.860	0.690–1.06	73	0.898	0.726–1.07	**
1st tarsal segment	59	0.125	0.105–0.144	66	0.131	0.112–0.15	***
2nd tarsal segment	59	0.261	0.224–0.310	66	0.277	0.222–0.321	***
Tarsal segment 2 / segment 1	59	2.09	1.86–2.32	66	2.11	1.88–2.37	
Count of setae on base of antennal segment 6	56	11.7	6–16	70	12.5	8–19	
Count of setae on 8th abdominal tergum	60	13.5	10–20	72	13.2	10–19	

TABLE 1. Continued.

Species and morph	*C. terminalis* apterae						
Host plant	*P. edulis*			*P. monophylla*			
Measurements	*N*	Mean	Range	*N*	Mean	Range	Sig.
Bradley's measure	16	1.36	1.23–1.50	20	1.28	1.10–1.40	**
Bradley's measure / Body	16	0.509	0.431–0.587	20	0.441	0.386–0.561	***
Rostrum 3	28	0.192	0.175–0.211	29	0.184	0.171–0.193	***
Rostrum 4	28	0.184	0.160–0.208	28	0.165	0.142–0.178	***
Rostrum 3 / Rostrum 4	28	1.05	0.933–1.16	28	1.12	1.02–1.26	***
Count of setae on rostrum 4	27	6.19	5–8	27	6.11	5–7	
Length of seta on antennal segment 3	25	0.053	0.027–0.090	27	0.055	0.032–0.083	
Length of seta on hind tibia	26	0.085	0.040–.141	29	0.080	0.040–0.144	
Length of seta on 5th abdominal tergum	28	0.055	0.013–0.094	29	0.064	0.033–0.112	
Hind tibia	26	1.87	1.51–2.09	28	2.09	1.71–2.41	***
Hind femur	26	1.11	0.93–1.22	29	1.23	.096–1.40	***
Tibia / Femur	25	1.68	1.47–1.85	28	1.69	1.61–1.78	
3rd antennal segment	25	0.422	0.295–0.473	27	0.452	0.367–0.507	*
4th antennal segment	25	0.163	0.121–0.209	27	0.187	0.130–0.222	***
5th antennal segment	25	0.217	0.167–0.255	27	0.240	0.203–0.478	***
Antennal segment 4 / segment 5	25	0.753	0.598–0.911	27	0.781	0.624–0.878	
1st tarsal segment	26	0.123	0.098–0.140	28	0.126	0.102–0.143	
2nd tarsal segment	26	0.267	0.204–0.313	28	0.285	0.246–0.308	***
Tarsal segment 2 / segment 1	26	2.18	1.94–2.40	28	2.27	20.3–2.57	**
Count of setae on base of antennal segment 6	23	6.9	4–10	27	7.3	5–10	
Count of setae on 8th abdominal tergum	27	12.4	10–18	28	12.9	10–18	

Species and morph	*C. wahtolca* apterae						
Host plant	*P. edulis*			*P. monophylla*			
Measurements	*N*	Mean	Range	*N*	Mean	Range	Sig.
Bradley's measure	13	1.43	1.36–1.60	41	1.57	1.37–1.82	***
Bradley's measure / Body	13	0.444	0.387–0.512	41	0.432	0.357–0.604	
Rostrum 3	16	0.190	0.166–0.210	76	0.216	0.194–0.235	***
Rostrum 4	16	0.153	0.138–0.163	76	0.167	0.147–0.187	***
Rostrum 3 / Rostrum 4	16	1.24	1.10–1.36	75	1.30	1.17–1.48	**
Count of setae on rostrum 4	16	5.94	4–8	74	6.49	4–8	*
Length of seta on antennal segment 3	15	0.078	0.056–0.104	76	0.104	0.070–0.139	***
Length of seta on hind tibia	16	0.096	0.072–0.112	72	0.120	0.076–0.152	***
Length of seta on 5th abdominal tergum	16	0.075	0.010–0.141	76	0.122	0.010–0.179	**
Hind tibia	16	2.37	1.99–2.91	70	2.82	1.57–3.32	***
Hind femur	16	1.41	1.15–1.70	72	1.69	1.27–2.01	***
Tibia / Femur	16	1.69	1.61–1.74	70	1.67	1.60–1.82	
3rd antennal segment	15	0.517	0.362–0.627	76	0.579	0.460–0.673	***
4th antennal segment	15	0.223	0.194–0.285	75	0.230	0.181–0.285	
5th antennal segment	15	0.275	0.173–0.346	74	0.328	0.259–0.394	***
Antennal segment 4 / segment 5	15	0.821	0.705–1.16	74	0.701	0.575–0.836	***
1st tarsal segment	16	0.129	0.115–0.141	70	0.139	0.107–0.154	***
2nd tarsal segment	16	0.258	0.233–0.289	70	0.289	0.253–0.328	***
Tarsal segment 2 / segment 1	16	2.00	1.83–2.14	70	2.09	1.83–2.59	**
Count of setae on base of antennal segment 6	13	8.6	5–12	69	8.5	5–11	
Count of setae on 8th abdominal tergum	16	19.8	14–28	74	26.8	12–38	***

TABLE 2. Principal components analysis. Sample size, percent of the total variance explained by the principal component, and mean of the component score for the individuals (*N*) in the sample; * indicates $P < 0.001$ in ANOVA; lack of * indicates $P > 0.05$.

Aphid species	Host species	*N*	1st component		2nd component		3rd component	
			% of variance	Mean	% of variance	Mean	% of variance	Mean
C. edulis alatae	*P. edulis*	30	36.0	–0.567*	14.7	0.113	13.0	0.063
	P. monophylla	28		0.608*		–0.121		–0.068
C. edulis apterae	*P. edulis*	48	46.5	–0.455*	15.1	0.051	9.0	0.405*
	P. monophylla	62		0.352*		–0.039		–0.314*
C. terminalis apterae	*P. edulis*	19	42.5	–0.516*	16.0	0.144	10.0	–0.641*
	P. monophylla	22		0.445*		–0.124		0.553*
C. wahtolca apterae	*P. edulis*	13	50.5	–1.287*	11.7	–0.403	7.2	0.309
	P. monophylla	63		0.266*		0.083		–0.064

and positively to the 2nd. Setal counts in *C. terminalis* and *C. wahtolca* did not follow any obvious pattern. In *C. terminalis* setal counts of the 4th rostral and 6th antennal segments contributed positively to the 4th principal component, and counts of the 8th abdominal tergum did not contribute appreciably to any of the components. In *C. wahtolca* there were negative and positive contributions to the 3rd component by setal counts of the rostrum and 6th antennal segment, respectively, and positive contributions to the 1st and 2nd components by the setal count of the 8th abdominal tergum (Fig. 3).

DISCUSSION

Aphid Size As It Relates to Host

Most nonrostral and nonsetal length measures were greater for all 4 groups of *Cinara* feeding on *P. monophylla* than for those feeding on *P. edulis* (Table 1). One pitfall of the univariate approach is the tendency to find significant differences in almost all measurements, as we have done here: Sokal (1962) found that almost every character differed significantly whether he compared gall-making *Pemphigus populitransversus* Riley between localities or between galls at the same locality. Also Sokal et al. (1980) and Sokal and Riska (1981) found as much morphometric variation between nearby populations of *P. populitransversus* and *P. populicaulis* Fitch as they did between more distant populations. Our sample sizes were not sufficiently large to enable comparisons between or within colonies on the same host species, and it is possible that had we done so, many univariate differences would have been found. However, we believe that the concordance of almost every nonrostral, nonsetal character across all 3 *Cinara* species is sufficient evidence to conclude that aphids feeding on *P. monophylla* are larger than those feeding on *P. edulis.*

Previous aphid PCA studies concluded that size played a major role in the 1st principal component (Wool 1977, Foottit and Mackauer 1990). The authors of one of the seminal works on principal components analysis (Jolicoeur and Mosimann 1960) also claimed that the 1st principal component is an indication of size if all eigenvectors are about equal and share the same sign. Despite this connection of the 1st principal component to size, however, all principal components are a combination of size and shape effects (Somers 1989, Sundberg 1989). Making a distinction between size and shape effects may even be hard to defend (Bookstein 1989), and Sprent (1972) reasoned that size and shape were largely inseparable, allometry being by definition change in shape concordant with change in size. For the purposes of this study, however, the strong, positive contributions to the 1st principal component by the 3 antennal segments, 4 leg segments, and body do corroborate the univariate analyses (Fig. 3).

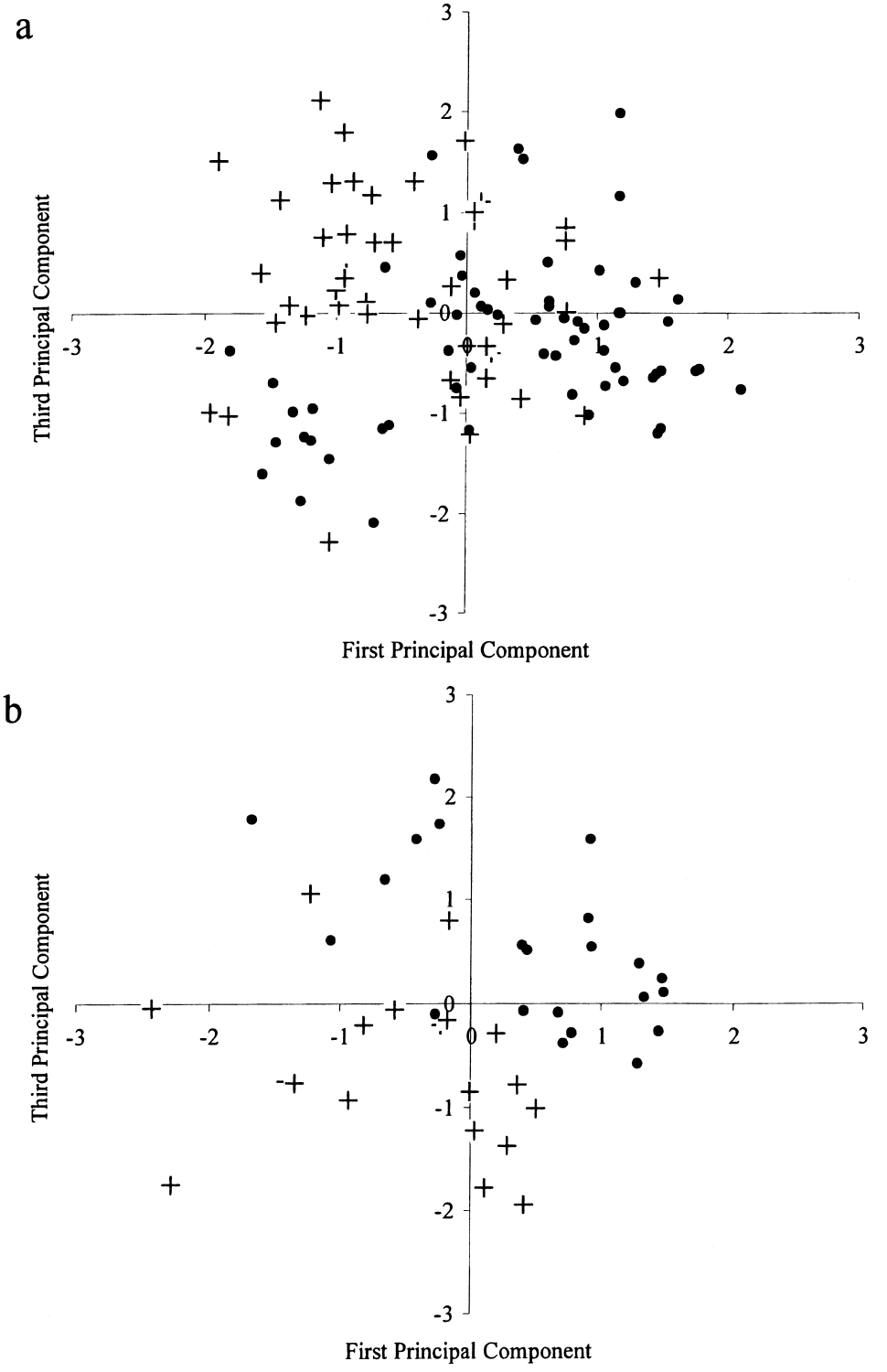

Fig. 2. 1st and 3rd principal components as calculated for individual aphids: (a) *C. edulis* and (b) *C. terminalis* apterae on *P. edulis* (+) and *P. monophylla* (•).

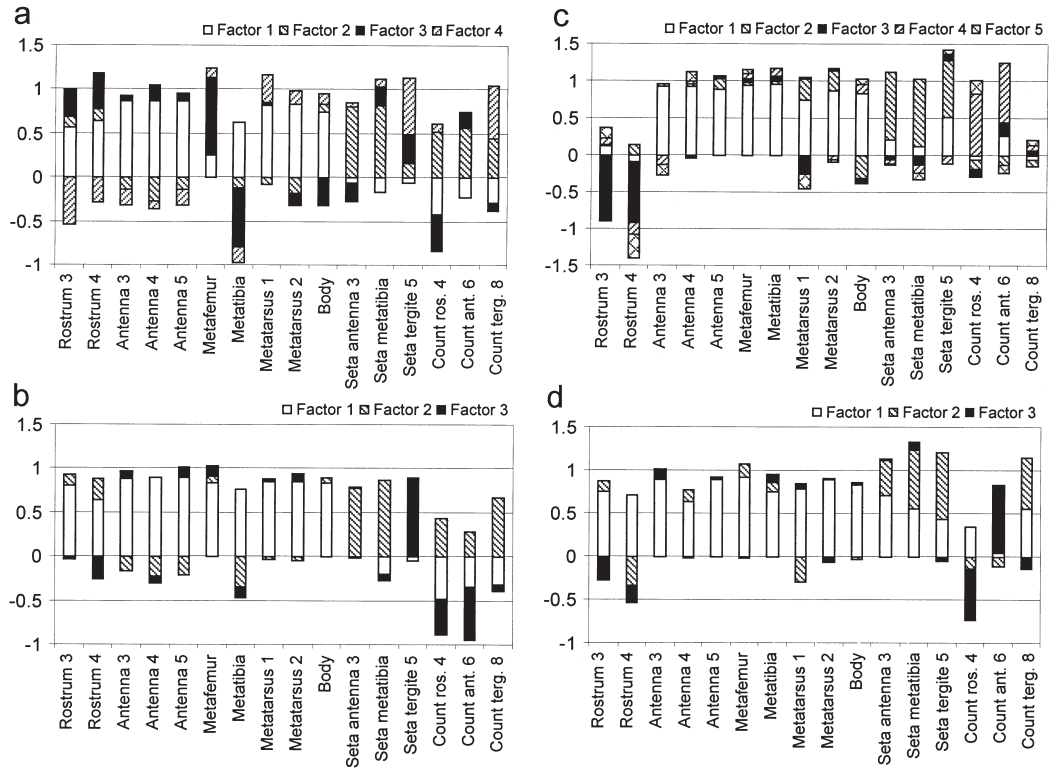

Fig. 3. PCA eigenvectors for morphometric characters: (a) *C. edulis* alatae, (b) *C. edulis* aptera, (c) *C. terminalis* apterae, and (d) *C. wahtolca* apterae.

Although the aphids studied are clearly larger when feeding on *P. monophylla*, we cannot determine whether this is a result of genetically based selective pressure or environmental induction (Thorpe 1976). Although methods have been developed to test for the masking of a genetic component to environment-phenotype correlation (Stinchcombe et al. 2002), our materials and methods do not answer the question of "nature versus nurture" with respect to aphid size. However, Via and Shaw (1996) documented selection for an increase in body size in *Acyrthosiphon pisum* (Harris) over the course of a single growing season, showing that aphid size can be genetically influenced and selected for. Thus, there is no a priori reason to discount selective pressure as the cause for the larger body size of *Cinara* species feeding on *P. monophylla* compared with those feeding on *P. edulis*.

There are any number of environmental factors that might cause *P. monophylla* to host larger aphids, whether by selection or induction. A higher nutritive quality of the host or weather-related phenomena may play a role: Wool (1977) found larger aphids in cooler climates, perhaps a means to reduce heat loss; a larger size would also result in a lower body surface area to volume ratio, thereby reducing the loss of water in drier environments.

Rostrum Length As It Relates to Host

Rostral measurements of *C. edulis* alatae and *C. edulis* and *C. wahtolca* apterae were greater in aphids feeding on *P. monophylla* than in those feeding on *P. edulis*, whereas those of *C. terminalis* apterae were smaller (Table 1). In *C. terminalis*, therefore, the rostrum followed the opposite trend as the rest of the body.

Multivariate analyses corroborate the univariate results. Lengths of the 3rd and 4th rostral segments of *C. terminalis* contribute most, and negatively, to the 3rd principal component

(Fig. 3c), in contrast to the positive 1st component contributions of those measurements in the other 3 groups (Figs. 3a, 3b, 3d). The big difference in the contribution of the rostral segments in PCA is likely the cause of the inverse relationship when the 1st and 3rd principal components of *C. edulis* and *C. terminalis* apterae are plotted on 2-dimensional graphs (Fig. 2): *P. edulis*–feeding *C. edulis* tend toward the negative and positive 1st and 3rd components (Fig. 2a, upper left of graph), whereas *P. edulis*–feeding *C. terminalis* tend toward the negative for both components (Fig. 2b, lower left of graph).

As rostrum length is not positively correlated with other characters in *C. terminalis,* there must be different influences acting on rostrum length and overall aphid size. Sokal (1952), studying *P. populitransversus,* also found rostrum length to be relatively independent of overall body size.

Aphid rostrum length has been tied to host plant properties, with respect to aphids feeding on pubescent hosts (Carter 1982, Moran 1986), and with respect to *Cinara* feeding on woody hosts (Bradley 1961, Voegtlin 1976). We cannot show conclusively a genetic correlation to host-related selective pressure affecting rostrum length in *Cinara,* but other evidence is suggestive. Moran (1986), because she studied variation between species, concluded that rostrum length in *Uroleucon* was under strong selective pressure and she even voiced a concern that environmentally or host-correlated characters, such as rostrum length (and tarsal length; Kennedy 1986, Moran 1986), may confound phylogenetic studies. Also, Favret and Voegtlin (2004) showed that speciation in *Pinus*–feeding *Cinara* is caused in part by host shifts, with new species developing on a new host at the same feeding site as on the ancestral host. Further, since feeding site and rostrum length are strongly correlated (Bradley 1961), we believe that rostrum length plays a constraining role as to possible patterns of speciation, and therefore is likely influenced by a strong genetic component.

Whether difference in rostrum length between *P. edulis*– and *P. monophylla*–feeding *C. terminalis* is due to evolutionary selection or environmental induction, the morphology of the host is probably the main cause. *Cinara terminalis* feeds on the growing tips of the branches of its host. Perhaps the bark of the shoots of *P. monophylla* is thinner (whether for developmental or environmental reasons) than that of *P. edulis.* The reverse might be true for *C. edulis* and *C. wahtolca* that feed on twigs and branches. Although we did not perform host-shift tests, based on our findings of rostrum size, it is likely that populations of any of the 3 species, were they moved to the other pinyon host, might suffer a loss of fitness related to rostrum length (Hawthorne and Via 2001).

We have posited the relative independence of rostrum length and overall size, our evidence largely coming from *C. terminalis.* However, in the case of the other 2 species, the 2 character suites may show correlated response (Nijhout and Emlen 1998). It is possible that selective pressure (or environmental induction) to increase the size of the rostrum may cause a concomitant increase in overall size. However, because all 3 species were larger on *P. monophylla,* it seems more likely that, if there is selective pressure, it favors larger aphids (as opposed to longer rostra) on *P. monophylla.* It is possible that a concomitant increase in rostrum length in *C. edulis* and *C. wahtolca* would be mal- or nonadaptive (Price and Langen 1992).

Taxonomic Comments

Foottit and Mackauer (1990) found no geographic population differences in *C. nigra* (Wilson) using PCA, Foottit (1992) found 3 morphologically distinct groups of *C. contortae* Hottes, but insufficient evidence to warrant separating them taxonomically, and Watson et al. (1999) described a new species of *Cinara* based on morphometric evidence. Caution precludes calling the *Cinara* on different pinyon species host-based races. Although there are clear size differences in the host-based populations of all 3 *Cinara* species, we cannot come to any firm taxonomic conclusions without showing that differences are genetically based (i.e., selected for). We have no other evidence for *C. edulis.* The opposing trends in rostrum and overall size in *C. terminalis* (in comparison with *C. edulis* and *C. wahtolca*) are compelling. Two known genetic clades of *C. terminalis* are geographically based, but the geography does not completely mirror host distribution (Favret and Voegtlin 2004). We performed morphometric analyses with the aphids in these 2 clades, but the results were less conclusive than the host-based results presented here. Further study of *C. terminalis* is required.

In the case of *C. wahtolca,* however, there is clear evidence of divergent lineages based on cytochrome oxidase 1 sequence data (Favret and Voegtlin 2004). Here we found that most examined characters showed significant differences based on aphid host affiliation, and differences were generally more pronounced than in *C. edulis* and *C. terminalis* (by comparing P-values). Three of the 5 ratios intended to control for size were also different, as well as all of the setal lengths (in contrast with the other species). Although the host-based morphometric differences between populations of *C. wahtolca* are largely based on size, as noted above, there is no reason to discount size as a critical component in morphometric differentiation of host races or even species. Given the genetic corroboration, we believe the populations of *C. wahtolca* found on the 2 species of pinyon are indeed different species, and we will describe the new species, feeding on *P. monophylla,* in a future paper.

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