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Asexuality and polyploidy in *Daphnia* from the tropical Andes

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Abstract

We assessed genetic variation at microsatellite loci within and among populations of the planktonic crustacean *Daphnia pulex* in 12 Bolivian Andean lakes, located above 4,000 m above sea level. Genetic analyses show that all populations consisted of obligately asexual lineages, a fact that was confirmed by observations from laboratory cultures. Moreover, microsatellite phenotypes indicate that these tropical lineages are polyploid. Levels of genetic diversity were comparable to those found in polyploid *Daphnia* from arctic regions, indicating a local origin rather than an accidental colonization from arctic regions. This is the first record of polyploid cladocerans in a tropical region. We suggest that their origination and abundance have probably been facilitated by the extreme environmental conditions in Andean lakes. Our analysis of multilocus genotype frequencies in relation to variation in environmental conditions indicates lineage sorting along a food availability and fish predation gradient.

One of the main themes in evolutionary biology has been the evolution of sex and the maintenance of sexual recombination in natural populations (Muller 1932; Kondrashov 1988; Maynard Smith 1992; Crow 1994; Butlin 2002). Asexual individuals in a sexual population have an important immediate reproductive advantage over sexual siblings, as they avoid investing in males, mate seeking and recognition, etc. (Maynard Smith 1971), but in the long term they suffer from mutational load and low evolutionary potential (reviewed in Goddard et al. [2005]). As such, there is an apparent conflict of interest between the population that benefits from sex and the individual that profits from the immediate gains of asexuality (Maynard Smith 1988).

In many taxonomic groups (e.g., ferns, higher plants, mollusks, worms, arthropods, fishes, amphibians, reptiles, etc.) sex has been abandoned by several offshoots of the evolutionary tree (Crow 1994). These asexual lineages can often live alongside sexual relatives. However, a disjunct spatial distribution pattern of sexual and asexual populations, termed geographic parthenogenesis, is frequently found in both animals and plants (Vandel 1928; Peck et al. 1998). Asexual organisms often dominate at high latitudes or in cold environments, while sexual organisms take over at lower latitudes or more temperate environments. In general, asexual organisms are believed to occur more in marginal, stressful, and resource-poor environments (reviewed in Peck et al. 1998).

Cladocerans are a group of crustaceans (Crustacea: Anomopoda + Ctenopoda) that almost invariably reproduce by cyclic parthenogenesis, a reproduction mode in which phases of asexual propagation are alternated with bouts of sexual reproduction. Sexual reproduction is always associated with the formation of resistant dormant eggs, which allow the organisms to bridge unfavorable periods. However, a few taxa have eliminated sex from this complex life cycle and produce dormant eggs parthenogenetically. The water flea *Daphnia pulex* Leydig (Crustacea: Anomopoda) and sister species are part of an intensively studied species complex, in which asexual lineages mainly occur at high latitudes and sexual strains dominate in temperate regions (Hebert and Crease 1983; Innes et al. 1986). The distribution of such obligate parthenogens in the Arctic has been studied in detail (e.g., Innes et al. 1986; Weider et al. 1999; Weider and Hobaek 2003). Remarkably, asexual diploid nonhybrid lineages of this species complex display a pattern of longitudinal, rather than latitudinal, segregation from their sexual counterparts: asexual populations predominate over much of the eastern part of North America, whereas cyclical parthenogens dominate in the west (Hebert and Finston 2001). Many of these asexual lineages have not, however, completely forsaken sex, as they can still produce males that can mate with sexual females, thereby regularly creating new asexual lineages in a strategy of contagious asexuality (Innes and Hebert 1988; Paland et al. 2005). Polyploid *Daphnia*, on the other hand, are invariably asexual lineages of hybrid origin (Dufresne and Hebert 1994) and are almost exclusively

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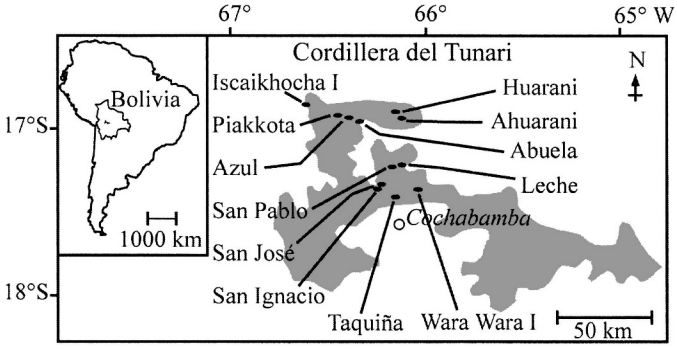

Fig. 1. Location of the sampled lakes in the Cordillera del Tunari, Bolivia.

found in the arctic. However, a few years ago a polyploid lineage of the *D. pulex* complex was documented from the southernmost part of Argentina (Adamowicz et al. 2002).

Asexual strains of the *D. pulex* complex often show strong associations between genotypes and environmental or biotic variables such as salinity, temperature, food, or predators (Loaring and Hebert 1981; Weider and Hebert 1987; Wilson and Hebert 1993), conforming to the frozen niche variation hypothesis that can be expected in asexual organisms (Vrijenhoek 1979). These associations indicate high dispersal rates and efficient genotype sorting in *Daphnia* (De Meester et al. 2002).

D. pulex s.l. have long been known from the tropical Andes (Harding 1955; Rey 1991), although they have hardly been studied in detail, ecologically or genetically. Even though many of the lakes in which they occur are situated in the tropical belt, the environmental conditions in these alpine lakes are very harsh. To some extent, they are reminiscent of the conditions in which asexual and polyploid lineages are typically found in arctic regions (Dufresne and Hebert 1998), with the main difference being that these tropical lakes are permanently ice-free and have primary production throughout the year. We studied genetic variation within and between 12 *D. pulex* populations from alpine Andean lakes in Bolivia. We had three main aims: (1) to determine the reproduction mode of these *Daphnia* populations using genetic markers; (2) to assess the genetic diversity within these populations; and (3) to relate the genetic composition of these populations to the geographical position of the lakes and their ecological characteristics.

Materials and methods

Study site and fisheries status—The polymictic and ice-free glacial lakes of the Cordillera del Tunari are located at altitudes ranging from 4,000 to 4,600 m above sea level (a.s.l.) in Bolivia (Fig. 1; Table 1). They have a glacial origin and were formed in the Pleistocene during the last

Table 1. Location, morphometric, and physico-chemical characteristics of the 12 study lakes of the Cordillera del Tunari, Bolivia. Values of DO, Temp, Cond, and pH are averaged over all depths, with standard error.*

Lake	Lat (°S)	Long (°W)	Altitude (m a.s.l.)	Area (km^2)	Maximum depth (m)	Secchi (m)	DO (mg L^{-1})	Temp (°C)	Cond ($\mu S\ cm^{-1}$)
Abuela	17.051	66.529	4,545	0.011	6.0	0.8	2.6±0.2	8.0±1.0	30.9±1.3
Ahuarani	17.055	66.248	4,369	0.100	8.5	3.5	5.1±0.1	10.9±0.3	22.3±0.2
Azul	17.052	66.536	4,513	0.061	16.0	0.8	5.6±0.4	11.0±1.4	19.1±4.8
Huarani	17.047	66.249	4,362	0.432	9.8	3.6	5.1±0.3	10.8±0.3	19.4±3.9
Iscaikhocha I	17.022	66.557	4,384	0.008	4.5	1.9	4.9±0.2	13.8±0.4	21.6±0.2
Leche	17.270	66.127	4,218	0.023	8.2	3.7	6.3±0.2	10.8±0.4	19.9±1.7
Piakkota	17.048	66.546	4,394	0.009	24.0	4.0	5.0±0.8	10.4±0.3	19.3±0.4
San Ignacio	17.248	66.195	4,313	0.037	7.6	2.7	5.4±0.1	11.3±0.1	26.8±0.2
San José	17.245	66.191	4,338	0.024	15.2	4.7	5.1±0.1	11.3±0.1	16.2±0.2
San Pablo	17.267	66.137	4,257	0.042	15.2	3.5	9.2±0.6	12.1±0.6	12.5±2.2
Taquiña	17.282	66.150	4,090	0.010	24.0	7.9	6.6±0.3	11.5±0.6	20.1±6.5
Wara Wara I	17.293	66.124	4,000	0.021	15.0	1.6	6.9±0.3	11.7±0.7	20.8±0.6

Lake	pH	DOC (mg L^{-1})	N (mg L^{-1})	P (mg L^{-1})	Chl *a* ($\mu g\ L^{-1}$)	Fish (SFE)
Abuela	5.4±0.1	3.86	0.30	0.28	2.24	0
Ahuarani	6.6±0.05	2.76	0.03	0.02	1.50	35
Azul	6.3±0.3	1.52	1.83	0.25	12.90	Reg. stocked
Huarani	6.6±0.3	2.28	0.03	0.1	2.80	57
Iscaikhocha I	7.6±0.2	NA	0.03	0.65	4.60	163
Leche	6.1±0.4	3.49	0.01	0.02	2.00	0
Piakkota	5.8±0.2	1.04	0.03	0.1	3.50	4
San Ignacio	6.7±0.2	3.2	0.07	0.03	2.70	0
San José	6.5±0.2	3.44	0.03	0.1	6.20	0
San Pablo	5.8±0.1	1.9	0.02	0.02	0.40	0
Taquiña	5.9±0.23	1.58	0.26	0.33	1.10	0
Wara Wara I	7.3±0.3	NA	0.08	0.35	3.80	Reg. stocked

* Lat, latitude; Long, longitude; DO, dissolved oxygen; Temp, temperature; Cond, conductivity; a.s.l., above sea level; DOC, dissolved organic carbon; N, nitrogen; P, phosphorus; Chl *a*, chlorophyll *a*; SFE, standardized fishing effort; Reg. stocked, regularly stocked, but not sampled.

retreat of the glaciers (Hutchinson 1957). All are located above the tree line (ca. 3,900 m a.s.l.) but well below the permanent snow line (ca. 4,800 m a.s.l.). The lakes are defined as cold polymictic tropical lakes (Hutchinson 1957), but the climate is alpine, with November isotherms around the tree line of ca. 11°C and July isotherms of ca. 6°C (Snow 1976). A detailed account of the limnology of the lakes is given in Aguilera et al. (2006*b*). The lakes were originally fishless, but beginning in 1940 rainbow trout (*Oncorhynchus mykiss*) was introduced in most of them (Dejoux 1986).

Sampling—During February 2003 and 2004 qualitative zooplankton samples were collected by vertical hauls with a conical tow net (25-cm diameter, 100-μm mesh size) in 12 Andean lakes, covering the whole depth range of the lake. Freshly caught animals were preserved in 100% ethanol for genetic analysis. In five lakes (Abuela, Leche, Piakkota, San Ignacio, and Wara Wara 1) we also included dormant egg banks by sampling offshore surface sediments from a boat using an Ekman grab. Subsamples of the upper 5 cm of sediment were taken and pooled and stored in the dark at 4°C. In the laboratory, ca. 150 g of sediment was washed through a 150-μm sieve. The residue was scanned at ×20–50 magnification for *Daphnia* ephippia (chitinized resistant capsules that contain dormant eggs). Ephippia belonging to the *D. pulex* complex were picked out and stored in the dark at 4°C.

From each lake we assessed physical, chemical, morphometric, and biotic variables (Table 1). Water temperature and dissolved oxygen, conductivity, and pH were measured at a single point 1 m below the surface and at increasing depths, with 1-m intervals, using the WTW OXI 330i, WTW Cond 315i, and WTW pH 330i devices (Hoskin Scientific). The maximum depth of each lake was recorded with a Humminbird Fishfinder 535 Portable echosounder (Techsonic Industries). Water transparency was measured using a standard 20-cm–diameter Secchi disc. Nutrients (nitrates and phosphates) and chlorophyll *a* (Chl *a*) were quantified from water samples taken at 1 m in depth. For Chl *a*, 200 mL of water was filtered onto a Whatman GF/F filter, and content was determined from acetone extracts using the spectrophotometric method of Nusch (1980). Phosphate and nitrate analyses followed the standard methods of the American Public Health Association (Eaton et al. 1988). Information on the presence of fish was obtained from local residents. In addition, in all lakes except Azul and Wara Wara I, a standardized fishing effort was carried out, using four floating gillnets (length of each net: 30 m; height: 3 m) in each lake during 20 h. The nets had different meshes (8, 10, 20, and 30 mm) and were attached to each other.

Genotyping—D. pulex specimens were picked out under a stereodissection microscope and transferred to 200-μL content microfuge tubes in 100 μL proteinase K-buffer [16 mmol L^{-1} $(NH_4)_2SO_4$; 67 mmol L^{-1} Tris-HCl, pH 8.8; 0.01% Tween-20; 7% DTT (dithiothreital); and 0.5 mmol L^{-1} proteinase K]. Dormant eggs were treated similarly in 30 μL buffer. Samples were incubated at 56°C for 1 h followed by 10 min at 95°C and 2 min centrifugation. Samples were stored at −20°C. In order to provide a reasonable estimate of the total clonal diversity per population, 30 individuals per zooplankton sample were subjected to microsatellite analysis at four microsatellite loci: Dp183, Dp464, Dp496, and Dp502 (Colbourne et al. 2004).

Polymerase chain reaction (PCR) was performed in multiplex. The total reaction volume (12 μL) consisted of 1× PCR buffer (Silverstar™, Eurogentec), 1.5 mmol L^{-1} $MgCl_2$, 200 μmol L^{-1} of each DNTP (deoxyribonucleotide triphosphate), 0.2 μmol L^{-1} of each primer, 1 μL of template desoxy-ribonucleic acid (DNA), 0.25–0.5 u *Taq* polymerase, and ultraviolet (UV)–sterilized mQ-H_2O. PCR amplifications involved a denaturing step of 5 min at 95°C, followed by 30 cycles of 17 s at 95°C, 17 s at 55°C, 17 s at 72°C, and a final elongation of 7 min at 72°C. Dormant eggs preserved in the sediment of four lakes were analyzed similarly. PCR products were separated and visualized on an ABI 3130 capillary sequencer (Applied Biosystems); samples were always run in parallel with a sizing standard (supplied by the manufacturer). Allele sizes were assessed with Genemapper 3.7 (Applied Biosystems). Clonal richness and diversity were calculated using the method of Weider et al. (1999).

Breeding system investigation—Breeding system was investigated by hatching decapsulated dormant eggs from the sediment of two lakes and culturing the offspring according to the method of Cousyn and De Meester (1998). Seven independent clones of each investigated population were followed for several generations, until ephippia with dormant eggs were produced. These were analyzed at four microsatellite loci, as described above. Sexual genotypes should show mendelian segregation of alleles in dormant eggs, as these latter alleles are produced sexually, whereas the genotypes of asexual *Daphnia* should be identical in ephippial eggs and parthenogenetic specimens.

Ploidy-level investigation—Although the most straightforward way of assessing ploidy level in *Daphnia* is by Feulgen staining and measuring DNA content optically in individual cells from tissues that do not display endopolyploidy (e.g., Adamowicz et al. 2002), the presence of more than two alleles per microsatellite locus is also indicative of an increased ploidy level. We considered individuals with three to four alleles per locus to be polyploid. A number of individuals and genotypes only had diploid microsatellite phenotypes (i.e., a maximum of two alleles per locus) and were subjected to an allele dosage analysis (Jenneckens et al. 2001) to indicate whether they were diploid or polyploid. The basic assumption behind an allele dosage approach is that within a single PCR, alleles of the same locus are amplified quantitatively in relation to the copy number in which they occur in the nucleus of each cell, for example, when only two alleles are present (say, alleles A and B). This should allow for the discrimination of tetraploid AAAB and ABBB phenotypes from diploid AB or polyploid AABB phenotypes, in a manner similar to that in which unbalanced staining patterns of allozyme loci are

sometimes used for this purpose (e.g., Adamowicz et al. 2002).

Allele dosage was performed on microsatellite phenograms created by Genemapper 3.7 using the relative peak sizes in each individual PCR amplification. To account for differences in amplification intensity between PCRs, allele sizes were standardized to the allele with the smallest peak size for each PCR and locus, and relative sizes of all alleles per locus were averaged for each genotype. Finally, microsatellite phenotypes were translated into absolute genotypes according to their putative ploidy level (diploid or tetraploid), inferred from the allele dosage results.

Genetic differentiation between populations in relation to geographic distances and lake characteristics—Early in the analyses, it became clear that all studied populations seemed to consist of asexual lineages. As the genetic properties of such lineages remain relatively constant across generations, lineages can be considered as analogues of species and populations as communities of species. We therefore relied on community ecological data analysis methods to assess genetic differentiation between lake populations and to relate population genotype composition to lake characteristics. We related patterns of among-population differentiation to the spatial distribution of lakes with Mantel correlation analysis. We calculated the Spearman rank correlation coefficient between two triangular matrices: one with the Bray–Curtis dissimilarity indices of all pairwise combinations of lakes based on the multilocus genotype (MLG) composition of *D. pulex* and one with the geographic distances between lakes. The level of significance of the association between the two matrices was assessed with 999 permutations, implemented in the RELATE routine of the software package Primer 5 (Primer-E).

Patterns of association between ecologically relevant variables (Table 1) and MLGs from each population were tested in a multivariate framework using Canoco (version 4.5). The test was run once with only the zooplankton data and once with the pooled zooplankton plus sediment samples. We applied centralization and standardization of environmental variables to allow for comparison of the estimated regression coefficients in common units. MLG compositions (frequencies) were square-root transformed. Canonical correspondence analysis (CCA) with forward selection of all environmental variables and 499 Monte Carlo permutations was applied, as a detrended correspondence analysis had indicated a predominance of unimodal species responses (Lepš and Šmilauer 2003). To check for multicollinearity among explanatory variables, a correlation analysis was applied.

Results

Genetic variation—In total, 15 microsatellite alleles were found over four loci in 358 individuals. All loci were polymorphic. The overall number of alleles per locus varied from two in Dp464 to six in Dp496. A total of 14 MLGs were found in 12 lakes and their sediment samples (Table 2). All genotypes were heterozygote in at least two loci (Table 3). Nine lakes were dominated (>90%) by a single MLG, while only three lakes contained more than two MLGs, with a maximum of five MLGs in Wara Wara 1, of which two were exclusively in the sediment sample (Table 2). MLG3 was by far the most abundant multilocus genotype, occurring in six lakes and representing 23% of all individuals. Seven genotypes (MLG6–MLG8 and MLG10–MLG13) were represented by only one individual. Average clonal richness and diversity were 1.81 ± 0.24 (mean $\pm$ standard error [SE]) and 1.28 ± 0.11, respectively, when considering only zooplankton samples. When the four sediment samples were included as well (and were pooled with the zooplankton samples), these values increased to 2.17 ± 0.37 and 1.41 ± 0.15, respectively (Table 1).

The MLG composition of active communities differed strongly from that of the dormant egg communities recovered from sediment samples. As we failed to find ephippia of *D. pulex* in Abuela, genetic analyses of ephippia were limited to lakes Leche ($n = 7$), Piakkota ($n = 25$), San Ignacio ($n = 13$), and Wara Wara I ($n = 24$). Two genotypes (MLG5 and MLG14) were exclusively found in sediment samples (Table 2). MLG5 was even the sole genotype in the sediment of San Ignacio and was not found elsewhere. The zooplankton of San Ignacio showed no trace of MLG5, consisting only of MLG3. Similarly, MLG3 was the only genotype in the zooplankton of Piakkota, while the sediment sample of this lake also contained MLG14. The sediment sample of Wara Wara 1 had a similar genotype composition to that from Piakkota, dominated by MLG3 and supplemented with MLG14, but it also displayed one individual with MLG2 (which was otherwise confined to Azul). The zooplankton sample from Wara Wara 1, however, harbored three different genotypes, two of which were absent from Piakkota. Only in Leche was the composition of sediment and zooplankton samples similar: both contained only MLG4. This genotype was restricted to this lake and the neighboring lake San Pablo.

Breeding mode assessment—The occurrence of fixed heterozygosity (i.e., when a whole population consists of a single heterozygote genotype) is often considered a strong indication for obligate parthenogenesis (Hebert and Crease 1983). We found six populations (zooplankton samples) that displayed fixed heterozygosity, while three other populations were nearly fixed (Table 1). We only observed a limited number of MLGs, and seven of these were found in more than one lake. In the four lakes in which sediment samples were assessed as well, we found either fixed heterozygosity in the sediment sample or MLG overlap with the zooplankton. These observations strongly conflict with expectations under mendelian segregation following sexual reproduction and thus strongly indicate that all populations studied here consist of asexual strains.

Fourteen clonal cultures originating from ephippial hatchlings of Wara Wara I ($n = 7$) and Piakkota ($n = 7$) were established. Surprisingly, two out of seven animals that hatched from Piakkota produced ephippial (dormant) eggs in their very first clutch (in the absence of males). One

Table 2. Multilocus genotype (MLG) frequencies per lake.*

Lake	MLG														CR	CD
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
Abuela			1												1	1.00
Ahuarani	0.93					0.07									2	1.15
Azul		0.57						0.03	0.4						3	2.06
Huarani	0.94						0.06								2	1.13
Iscaikhocha I			1												1	1.00
Leche Z				1											1	1.00
Leche S				1											1	1.00
Leche P				1											1	1.00
Piakkota Z			1												1	1.00
Piakkota S			0.88											0.12	2	1.27
Piakkota P			0.92											0.08	2	1.17
San Ignacio Z			1												1	1.00
San Ignacio S					1										1	1.00
San Ignacio P			0.55		0.45										2	1.97
San José									1						1	1.00
San Pablo				0.97									0.03		2	1.06
Taquiña			0.34							0.03	0.6	0.03			4	2.09
Wara Wara 1 Z	0.52	0.03	0.45												3	2.11
Wara Wara 1 S			0.75								0.04			0.21	3	1.64
Wara Wara 1 P	0.29	0.02	0.58								0.02			0.09	5	2.31
Average P (SE)															2.17 (0.36)	1.41 (0.15)
Average Z (SE)															1.83 (0.29)	1.30 (0.13)

* S, sediment sample; Z, zooplankton sample; P, averaged over Z and S; CR, clonal richness; CD, clonal diversity; SE, standard error.

of them even produced ephippial eggs for three consecutive moult cycles. All other individuals ($n = 12$) first formed subitaneous eggs that developed into juvenile female *Daphnia*. After a variable number of moult cycles and population growth, all cultures eventually switched to the production of ephippial eggs. Ephippia could be formed in the absence of males in all cultures, proving that the lineages are obligately asexual. Surprisingly, males were produced when cultures became very dense, showing that the genotypes have retained the capacity to produce males. It is unclear, however, whether these males are still functional.

Ploidy level assessment—Ten MLGs (MLG5–MLG14) showed three to four alleles in at least one locus, indicating that at least these genotypes are polyploid, and likely tetraploid (Table 3). Based on this criterion alone, at least nine lakes contained polyploid genotypes, although sometimes only in the sediment samples (Table 2). In addition, each of the four remaining genotypes with diploid

Table 3. Supposed complete tetraploid genotypes derived from allele dosage analysis for all genotypes. Putative null-alleles are indicated with a zero. Allele sizes in nucleotides.

MLG No.*	Locus															
	Dp183				Dp464				Dp496				Dp502			
1	111	111	111	111	153	153	153	153	202	202	205	205	142	142	142	151
2	111	111	111	111	149	153	153	153	202	202	205	205	142	142	142	151
3	111	111	111	111	149	0	153	153	205	205	208	208	142	142	142	151
4	111	111	113	113	149	0	153	153	199	199	211	211	148	148	148	151
5	107	0	111	113	149	149	0	153	202	202	202	208	142	142	142	142
6	111	111	111	111	153	153	153	153	202	202	205	205	142	142	145	151
7	111	111	111	113	153	153	153	153	202	202	205	205	142	142	145	151
8	111	111	111	111	149	0	153	153	202	202	205	205	142	142	145	151
9	111	111	113	113	149	0	153	153	202	202	211	211	142	142	145	151
10	111	111	111	113	149	0	153	153	205	205	0	208	142	142	145	151
11	111	111	113	113	149	0	153	153	202	202	217	217	142	142	145	151
12	111	111	111	111	149	0	153	153	205	205	208	208	142	142	148	151
13	111	111	113	113	149	0	153	153	199	199	211	214	148	148	148	151
14	111	111	113	113	149	0	153	153	205	205	208	208	142	145	148	151

* MLG, multilocus genotype.

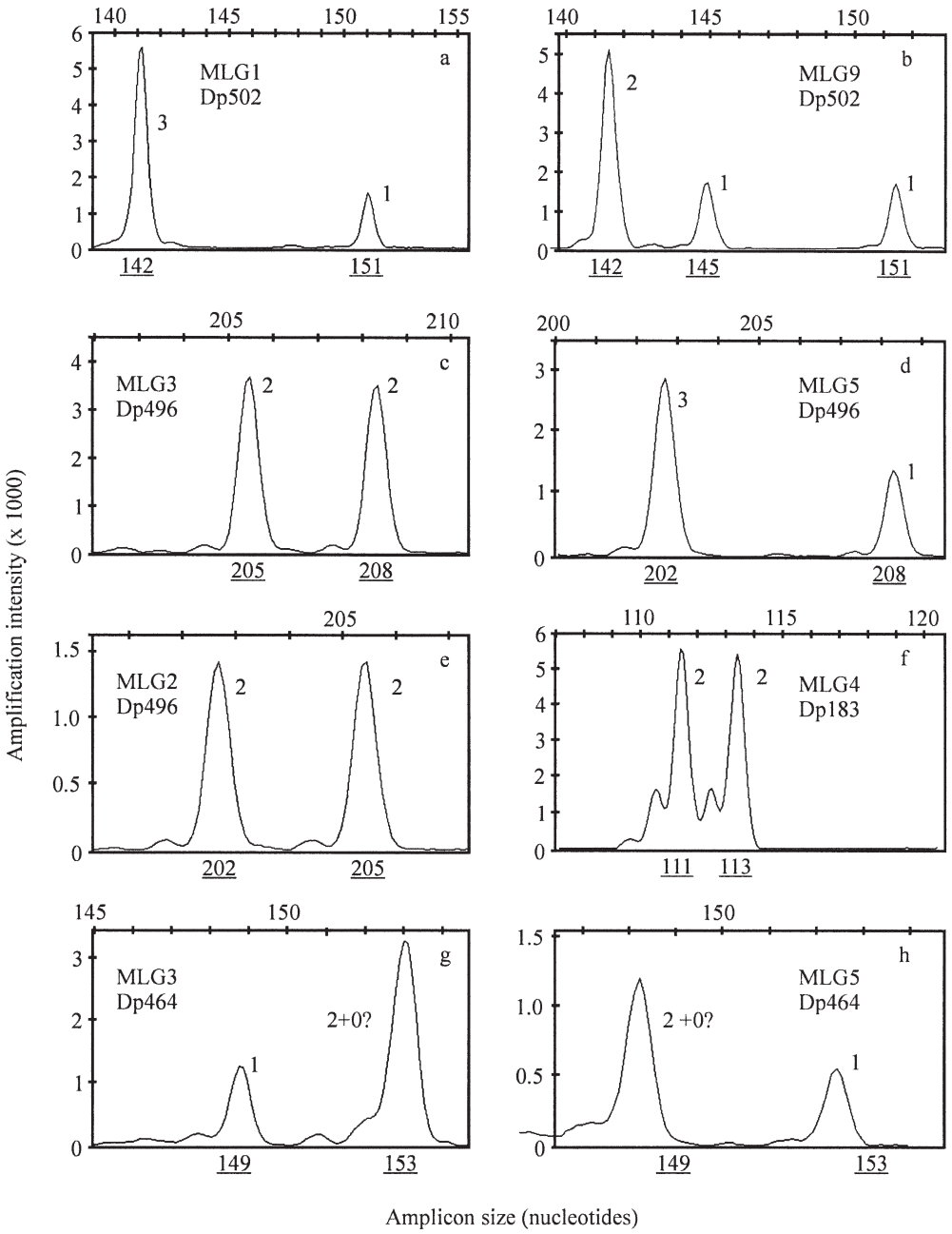

Fig. 2. Examples of microsatellite phenograms at different loci and MLGs, indicating allele dosage differences (a–h). Underlined numbers represent the allele names. Numbers at the peaks indicate presumed copy number of each allele per nucleus, assuming tetraploidy. "0?" represents a putative null-allele.

phenotypes showed unequal allele dosage patterns in at least one locus, with consistent allele dosage ratios (Fig. 2; Table 4). For example, locus Dp502 tended to show a 3.1 : 1 ratio between the two alleles in MLG1–MLG4, which is in accordance with a tetraploid configuration with three and one copies of each allele (Fig. 2a; Table 4). At locus Dp496, these four MLGs displayed equal intensities at the two observed alleles (Table 4), indicating that both alleles were present in equal copy numbers. However, some allele dosage patterns were less consistent, with a 3 : 1 or 1 : 1 ratio, as in Dp464. On average, this locus showed a 1.9 : 1 ratio between its two alleles in most genotypes, which may have been caused by the presence of a null-allele (nonamplification of one allele; Fig. 2g,h; Table 3). Similarly, in MLG5, ratios of the three alleles found at dp183 were all close to 1 (1.2, 1.1, and 1.0), which may be due to the presence of a null-allele.

Nevertheless, the combined results indicate that all genotypes found in the studied Bolivian lakes are polyploid. This is further supported by the observations that populations dominated by MLGs with diploid phenotypes (MLG1–MLG4) also occasionally contain genotypes that

Table 4. Average allele dosage and standard deviation at multilocus genotypes (MLGs) with diploid microsatellite phenotypes at the four studied loci.*

MLG No.	Dp183	Dp464	Dp496	Dp502
1	X	X	202/205: 1.06±0.08	142/151: 2.72±0.50
2	X	153/149: 2.47±0.48	202/205: 1.02±0.06	142/151: 3.43±0.71
3	X	153/149: 1.75±0.64	205/208: 1.17±0.11	142/151: 3.12±1.21
4	111/113: 1.19±0.21	153/149: 1.75±0.19	199/211: 1.29±0.11	148/151: 3.05±0.67

* X, only one allele present.

are nearly identical, except for the presence of a third allele in one of the loci (e.g., Huarani: MLG1 and MLG7; Azul: MLG2 and MLG8, Taquina: MLG3 and MLG12; San Pablo: MLG4 and MLG13; Table 3). Most likely, these new alleles originated through a single mutation of one allele that was present in multiple copies in a polyploid genotype (e.g., compare Fig. 2a with Fig. 2b).

Environmental variation and MLG distribution—The studied lakes varied from 4.5 to 24 m in maximum depth, all with very low conductivity levels and an acidic to neutral pH (Table 1). Phytoplankton biomass was generally low, and most lakes were therefore also very transparent. No floating or submerged vegetation was observed during the sampling period. Average temperature in the lakes ranged from 10.8°C to 13.8°C. In six of the sampled lakes (Azul, Wara Wara I, Piakkota, Iscaikhocha I, Ahuarani, and Huarani), we caught fish with the gillnets or local people informed us that the lakes had been stocked regularly. In five lakes (Taquiña, Abuela, San José, San Ignacio, and San Pablo), we failed to catch fish, indicating that these lakes were fishless or contained only low fish densities.

Canonical correspondence analysis indicated that Chl *a* ($p = 0.0006$) and fish ($p = 0.038$) explained 31% of the variation in MLG composition in the lakes (Fig. 3). Fish and Chl *a* were not correlated significantly to each other. Only phosphorus and nitrogen, altitude and dissolved oxygen, depth and dissolved organic carbon showed significant correlations. Even when the most common genotype (MLG3) was omitted from the analysis, both fish and Chl *a* were still marginally significant in a CCA forward selection procedure ($p = 0.062$ and 0.064, respectively), indicating that the correlation between genotype composition and environmental variables is not solely due to a single abundant genotype. Inclusion of MLGs that were only found in the sediment samples (using the pooled zooplankton plus sediment data sets) did not affect the outcome of the test. We found no significant relation between MLG composition of the lakes (Bray–Curtis index) and geographic distance ($\rho = 0.025$, $p = 0.48$).

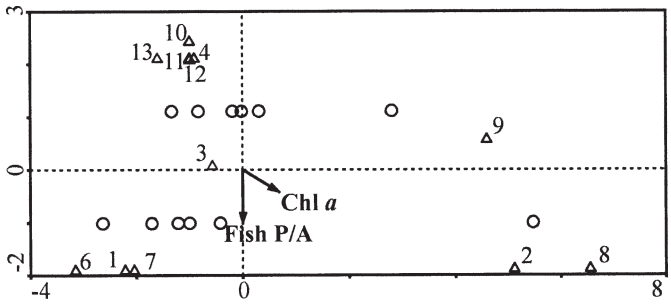

Fig. 3. CCA triplot showing the position of the sampled lakes (circles), genotypes (triangles with MLG number) in relation to fish presence/absence, and Chl *a* concentration.

Discussion

Tropical asexuality and polyploidy—Sexual recombination of genetic material has been a key innovation in evolution, as it allows rapid adaptation to changing environmental conditions, including biotic arms races (Lively 1996; Waxman and Peck 1999). In very predictable but marginal environments, however, the advantages of sex may be reduced, as adaptations to these environments may require co-adapted gene complexes, which are broken up by sexual recombination. Our results clearly show that the studied Bolivian populations of *D. pulex* consist entirely of asexual strains. Despite resemblances in summer temperature and trophy level, tropical alpine lakes strongly differ from arctic lakes with respect to seasonality and associated primary production, as they lack seasonal ice cover, while sun drives primary production all year round, with two maxima of solar radiation. This is in strong contrast with the seasonal blackouts in polar regions. At first sight, it therefore seems remarkable to find obligately asexual populations of *Daphnia* in tropical, albeit alpine, lakes. However, this fits rather well with the general pattern of geographic parthenogenesis, in which the relative abundance of asexual taxa increases with latitude but also with altitude. The studied Bolivian lakes were characterized by very little habitat structure and diversity, as they largely lack submerged vegetation and are cold and oligotrophic, showing relatively little variation in temperature, conductivity, and pH (Table 1). The overall pattern that asexual lineages tend to dominate in marginal and stressful habitats (Peck et al. 1998) seems to hold also for these populations. Moreover, the geographic distribution of many asexual taxa coincides remarkably well with regions that have a history of glaciation or desertification during the last glacial maxima (Kearney 2005), which includes the arctic as well as large parts of the Andes and Patagonia (Hooghiemstra 1989; Ray and Adams 2001). Taken together, this indicates that the selective forces favoring asexuality in arctic and tropical alpine regions are similar.

Ten out of 14 MLGs of *D. pulex* were clearly polyploid, as evidenced by their microsatellite phenotypes, with up to four alleles per locus. The remaining four genotypes had

diploid phenotypes but were likely also polyploid, as indicated by the allele dosage analysis: each of these genotypes displayed unbalanced allele dosage in at least one locus, which is concordant with a tetraploid configuration. Although polyploidy is exceptional within the genus *Daphnia*, it occurs in several members of the *D. pulex* complex, such as *Daphnia middendorffiana, Daphnia tenebrosa,* and American *Daphnia pulicaria* (Hebert 1995; Colbourne et al. 1998). In North America and Europe, polyploid *Daphnia* lineages are absent from temperate regions (Ward et al. 1994; Hebert and Finston 2001) and only become dominant north of the 10°C July isotherm (Adamowicz et al. 2002). Similar results have been obtained in the cladoceran genus *Bosmina* (Little et al. 1997). As a result, polyploidy in cladocerans has long been thought to be restricted to arctic regions. However, Adamowicz et al. (2002) found polyploid members of the *D. pulex* complex in Patagonia, where a cold temperate climate prevails (austral summer isotherms of 9–16°C). The present study shows that polyploid *Daphnia* even occur into the tropical belt of South America. With an isotherm of ca. 11°C during the warmest month, however, our data do corroborate earlier suggestions that polyploidy in *Daphnia* is adaptive in cold and stressful climates (Dufresne and Hebert 1998).

In arctic regions, melanic *Daphnia* lineages or species are regularly found (Hebert 1995), and this is believed to be an adaptation to elevated levels of UV irradiation in these regions. UV incidence in the high Andes is fairly high and comparable to that found in polar regions (Aguilera 2006), yet no melanic *D. pulex* were found in the studied Bolivian lakes. We did observe *Daphnia peruviana*, a highly melanized species endemic to the Andes, in one of the lakes, San Pablo. Moreover, *D. peruviana* occurs in shallow intermittent pools in the region (Coronel pers. comm.). Possibly the large depth of the studied lakes and the ability to migrate vertically (Aguilera et al. 2006*a*) provides sufficient protection against UV irradiation (Rhode et al. 2001), whereas *Daphnia* in shallow pools have to resort to photoprotective compounds like melanin to cope with UV stress.

Regional species and genetic diversity—The populations discussed in the present study are the only *Daphnia* observed in the studied lakes, with two exceptions: in San Pablo *D. peruviana* was found, and in Abuela ca. 15% of the individuals belonged to a species of the *Daphnia obtusa* complex. Allozyme and microsatellite results (Aguilera and Mergeay unpubl. data) indicate that this latter species is diploid and reproduces sexually, indicating that sexual and asexual *Daphnia* species coexist to a certain degree in these elevated Andean lakes. The only other species that is known from the regional species pool is *Daphnia inca*. Both *D. peruviana* and *D. inca* are mostly found in intermittent shallow pools (Coronel pers. comm.).

Within the *D. pulex* complex, our survey revealed a total of 14 MLGs in an area of ca. 1,500 km^2. Allozyme analysis (seven loci) on populations from the same lakes revealed a total of nine MLGs (Aguilera 2006). If MLGs represented by a single individual are omitted, allozyme analysis revealed a total of seven MLGs, compared to eight using microsatellites (Table 2), indicating that both marker types can detect clonal diversity to an equal degree. The regional clonal diversity found in our study region is much higher than the two allozyme MLGs found in 16 populations in southern Argentina, which covers an area that is 100 times larger (Adamowicz et al. 2002). Mean clonal richness and diversity per population in our study rather corresponded to values observed in Arctic *Daphnia pulicaria* (Weider et al. 1999). This high genetic diversity is at odds with the idea that South American polyploid lineages were lucky founders that recently dispersed from the North American arctic (Adamowicz et al. 2002). Our results indicate instead that the Bolivian lineages have long been present in South America.

Dormant egg banks are important reservoirs of genetic diversity of local populations, as they can contain viable dormant eggs that are several years, decades, or even a century old (Cáceres 1998; Mergeay et al. in press). As a result, active zooplankton populations are expected to represent a subsample of the diversity found in the dormant egg bank. Furthermore, a comparison between the genetic composition of dormant propagule banks and modern populations can provide information on recent changes in the genetic composition of populations. In the five lakes we examined for ephippia, only one (Leche) showed a similar genetic composition of the zooplankton and sediment samples. In Abuela, no ephippia of *D. pulex* were found, which may indicate that its sole genotype, MLG3, does not invest in dormant stages under certain circumstances but rather in population maintenance throughout the year (Gliwicz et al. 2001). Alternatively, the failure to find ephippia may be due to nonrandom deposition of ephippia (e.g., as a result of wind-driven aggregation of floating ephippia onto the shore of this lake). Nevertheless, ephippia of MLG3 were found in other lake sediments, showing that production of dormant eggs in this genotype is common. Similar differences in frequencies of occurrence between sediment and zooplankton samples were observed for MLG1 in Wara Wara 1 and MLG3 in San Ignacio (Table 2). These differences may be ascribed to a lack of production of ephippia by certain MLGs, to seasonal succession of genotypes, and/or to recent colonization events.

There were also lakes in which specific MLGs only occurred in sediment samples. The egg bank of San Ignacio indicates that MLG5 has persisted in this lake for a long time. In the zooplankton sample, however, we only found MLG3. Although this may reflect that MLG5 has disappeared from this lake, it may also result from a sampling artefact: indeed, although we tried to sample the whole water column of the lake, we may have missed the bottom centimeters, which often harbor large proportions of the *Daphnia* population (Aguilera et al. 2006*a*). These bottom-dwelling *Daphnia* may be of a different genotype than the specimens found in the pelagic zone.

Lineage sorting of tropical polyploid Daphnia?—In an attempt to relate the genetic composition of the studied *Daphnia* populations to environmental characteristics of the lakes, we found that Chl *a* and fish presence were significantly related to genotype composition of the lakes. These results add to those of earlier studies on asexual

Daphnia species in arctic regions, in which strong lineage sorting along environmental gradients (salinity, temperature, predation, etc.) has typically been observed (Weider and Hebert 1987; Wilson and Hebert 1993). Among the investigated explanatory variables, food availability, here quantified as Chl *a* content, can be expected to exert a strong selective pressure, especially in oligotrophic systems. Secondly, as *D. pulex* is the largest zooplankton species in the studied lakes and is prone to fish predation (Aguilera 2006), clones poorly adapted to fish predation will probably be eliminated rapidly in lakes stocked with trout. Although our study covered only a limited number of lakes, our results thus are suggestive of lineage sorting among polyploid asexual *D. pulex* strains along two ecologically important gradients, food availability and predation risk.

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