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Morphometric divergence of Robertsonian populations/species of *Mus:* A multivariate analysis of size and shape

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Summary. The morphological divergence of Robertsonian populations from the Rhaetian Alps (2n=26, 2n=24 and 2n=22) was investigated by multivariate analysis of shape components of the mandible and scapula. These shape components were obtained by a specifically designed multivariate procedure that detects and negates variation in general size. Whilst allozymes frequencies fail to effectively distinguish between Robertsonian populations the above multivariate morphometric procedures showed that the Robertsonian populations are clearly morphometrically distinct and that this morphometric divergence is cladistically congruent with the chromosomal evolution. Given appropriate comparative material, the karyotype of a mouse from these populations can be predicted from the shape of its mandible.

After the discovery that the Tobacco mouse[1] of Val Poschiavo had a 2n=26 karyotype[2] due to Robertsonian fusions of the ancestral (2n=40), acrocentrics many other Robertsonian populations of *Mus musculus* have been found. One of the best known of these is the system of 7 Robertsonian populations in the Rhaetian Alps investigated by Capanna[3] and his co-workers.

The chromosome arms can be identified by their T-G banding pattern and shared (uniquely derived) fusions can be recognized. This has allowed the phylogenetic relationships of these populations to be reconstructed and the process of stasipatric speciation to be investigated[3,4]. Robertsonian fusions tend to lead to reciprocal reproductive isolation between populations due to either hybrid sterility (as a result of failure of meiosis at the 1st spermatocyte), or to greatly reduced fertility in the heterozygote[3]. This process of speciation is thought to be quite rapid (5000 years) on the basis of archeological evidence[3] and the lack of isozyme divergence[5].

Whilst the existence of at least some of these chromosomally distinct populations of *Mus* has been known for some time a recent review of mouse morphometrics[6] revealed no serious attempt to investigate the morphological affinities of these Robertsonian populations.

This paper attempts to answer the following questions. Are the sympatric and parapatric Robertsonian populations morphologically distinct? If they are morphologically divergent, are the cladistic relationships congruent with those hypothesized on the basis of their chromosomal affinities?

4 populations (fig. 1) has been used in this study (represented by 60 specimens). The 3 Robertsonian populations from the Rhaetian Alps are as follows:

1. 2n=26, Upper Valtellina, 2. 2n=24, Upper Valtellina[7], 3. 2n=22, Orobian. The 2n=26 population, which is sympatric with the 2n=24 population, is the well known Tobacco mouse as it is chromosomally identical with the Val Poschiavo 2n=26 population. A population from Burano, South of Orbetello, Tuscany, (2n=40) was used for outgroup comparison.

The phylogenetic relationships of these populations, based on the shared, derived chromosomal fusions, is represented in figure 2.

Some Robertsonian populations (i.e. 24 and 26) do not differ by obvious external features readily recognizable to the human eye. Moreover, they have not yet been successfully distinguished by isozymes frequencies[5]. This is not particularly suprising as both approaches are inferior to multivariate morphometric analysis when it comes to distinguishing between closely related populations of mice[6,8,9]. The morphological character systems chosen for this study were the mandible and scapula since their features can be recorded quickly and accurately. A modified recording procedure[8] allowed us to record 13 mandible and 9 scapula characters by placing these bones on a photographic negative of mm graph paper (reduced 9 times) and viewing them down a binocular microscope (fig. 3).

Since mice grow throughout life it is not possible to distinguish between the influence of ontogenic growth and

any genetical/phenetical factor influencing size. Consequently, it is necessary to eliminate the influence of size if the population affinities are to be assessed[10]. Failure to do this will perturb the relative similarity of the population and possibly result in incongruence between character systems as in Berry et al.[11].

The influence of size can be negated by the appropriate bivariate or multivariate[10] methods. Since size is essentially a multivariate concept and all characters in this study are potentially size dependent, a multivariate approach was adopted. Moreover, between population differences will generaly obscure an assessment of size and shape unless the appropriate statistical procedures are followed. This is true of both bivariate and multivariate approaches and is explained geometrically by Thorpe.[10] Due to the above considerations the following procedure was adopted for negating the influence of size in the osteometric character sets.

The characters were converted to logs to render the relationships between them linear. The within-population covariance matrix was computed for each population independently and then pooled to give a pooled within-population covariance matrix. This matrix is real, symmetric and positive definate. It is not semidefinate unless an inadequate number of specimens is used in which case the procedure is inadvisable.

All the eigenvectors and roots are extracted from this matrix using a high-accuracy algorithm and the resultant component scores of the individuals from each group are computed. The eigenvectors loadings of each character are examined and the size vector detected. This vector is then excluded from further considerations and the component scores for the other nc - 1 vectors (where nc = number of characters) are then regarded as size-independent 'characters'. These orthogonal variables are in fact linear combinations of the original characters hereafter referred to as component variables in order to distinguish them from the original osteometric variables. A further explanation of the methodology and its rationale is to appear elsewhere.

The divergence between populations was assessed independently for mandible and scapula data sets using the components variables (minus the size component) as data for canonical analyses. Canonical analysis ordinates groups so that the between-group distance is maximized in relation to the within group variance. It is thus an optimal technique for displaying the morphometric divergence and relative similarity of the populations[10].

The cladistic relationships between the populations are investigated by computing the Manhattan distances between population means of the normalized component variables, minus the size component[13]. A Wagner network is then computed between population means[12] and is converted to a cladogram or Wagner tree by outgroup rooting with the 2n = 40 population from Burano, Tuscany.

It is evident that the Robertsonian populations are morpho-

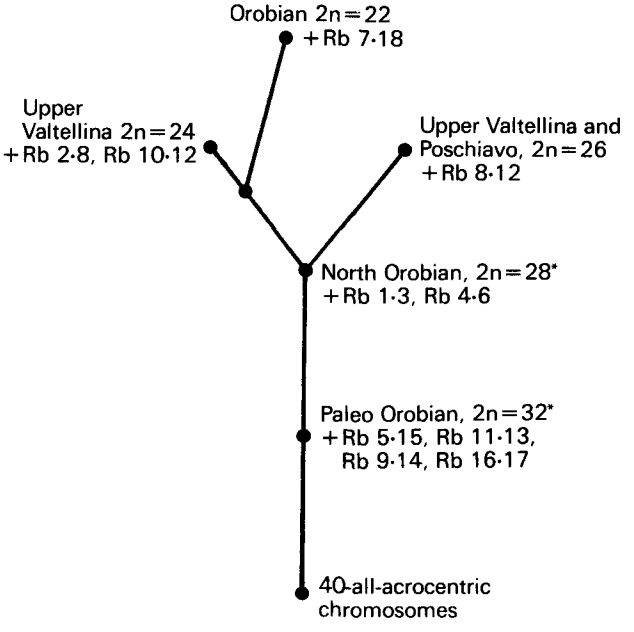

Figure 2. Hypothesized phylogeny of the Alpine populations based on chromosomal fusions. Novel Robertsonian fusions are indicated by + Rb followed by the code number of the chromosomal arms undergoing the fusion. Asterisks indicate hypothetical populations[3,7]. There are no reversal (fissions) hypothesized.

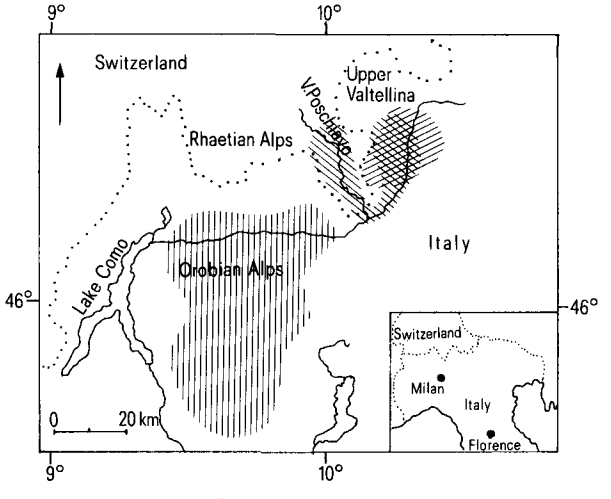

Figure 1. Range of Robertsonian populations. 2n = 22, Orobian population = vertical shading; 2n = 24, Upper Valtellina population = shading slopes up to the right; 2n = 26, Upper Valtellina and Poschiavo population = shading slopes down to the right.

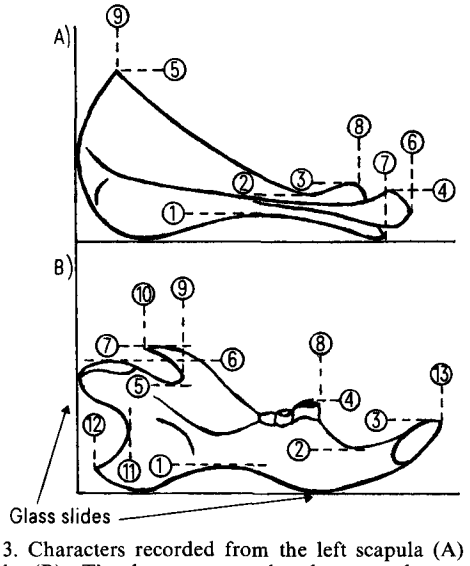

Figure 3. Characters recorded from the left scapula (A) and left mandible (B). The bones were placed on a photographically reduced (9 times) negative of mm graph paper and viewed down a binocular microscope. A constant position is achieved by placing the bones against fixed glass slides.

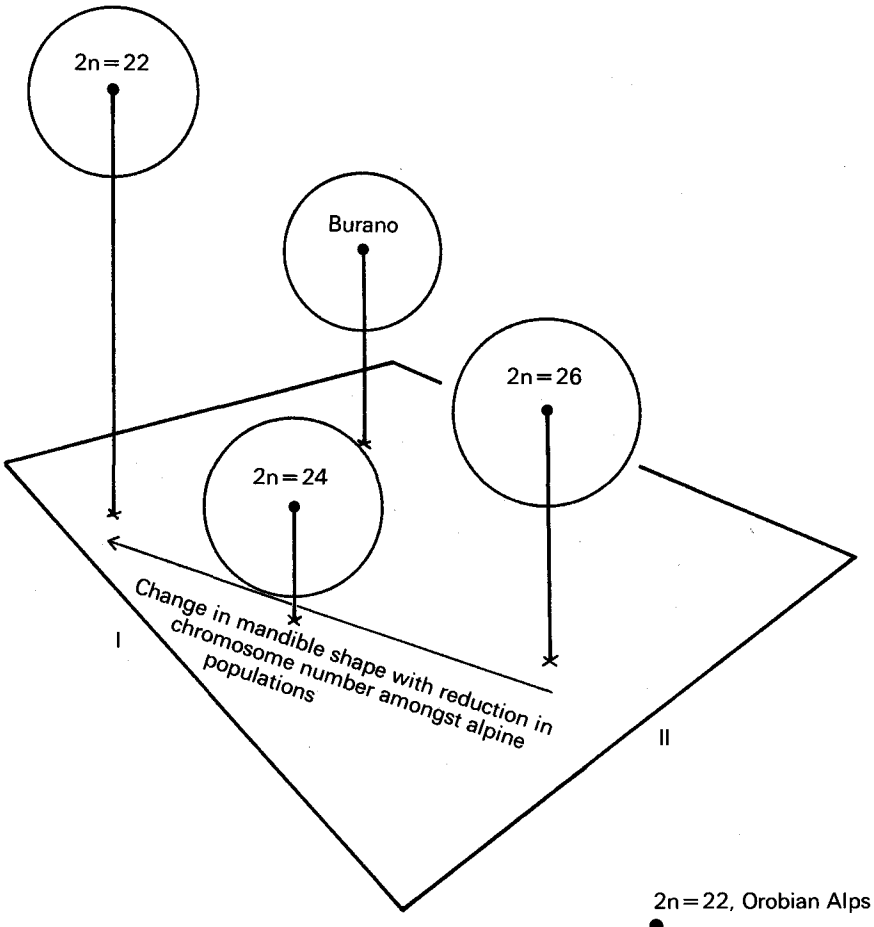

Figure 4. Canonical analysis of male mandible shape. In this 3D plot the 3rd and final canonical variate is the vertical axes and the probability circles around the group centroids have a radius of 2 SD. The Robertsonian populations are clearly discriminated and a morphological progression is evident which coincides with the evolutionary reduction in chromosome number amongst the Alpine populations.

logically distinct. Discriminant functions based on the component variables of mandible shape completely distinguish between the various populations. Since specimens can be classified into their correct Robertsonian populations using mandible shape, it is possible to accurately predict the karyotype of an individual on the basis of its osteology, given appropriate comparative material. Consequently, it should be possible to indicate the karyotype of museum specimens from their mandible shape. This may be of value in adding a historical perspective to range expansion of these Robertsonian forms.

Canonical analysis of the Robertsonian populations (fig. 4) not only indicates their morphometric distinctness but also their relative dissimilarity. A morphocline is apparent which is coincident with the evolutionary reduction in chromosome numbers amongst the Alpine populations (26-24-22). This analysis, based on males and using component shape variables derived from all 13 mandible characters is supported by parallel analyses with fewer characters and more specimens and also be canonical analysis of component variables derived from scapula characters 1,2,3,4,5,6,7,8 and 9. Canonical analysis of female mandibula characters also supported these analyses.

The cladistic relationships between populations based on mandible shape (fig. 5) indicates that populations with more Robertsonian fusions (i.e. 2n=24 and 22) share a more recent common ancestor than either do with the population with fewer fusions (2n=26). Thus, there is congruence between the independently derived cladistic hypotheses based on karyotype and mandible shape. These hypotheses therefore corroborate one another.

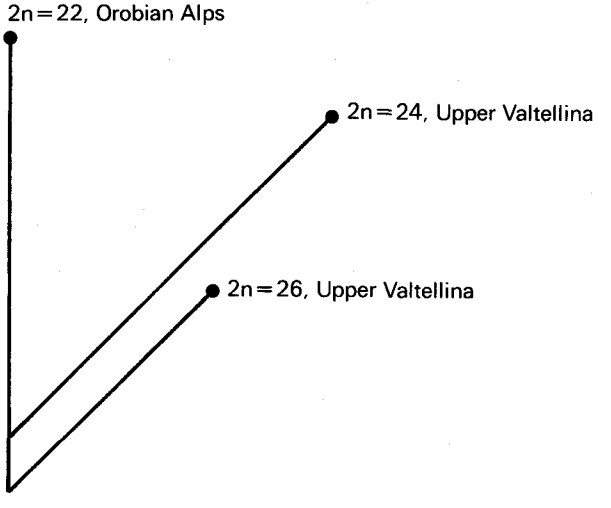

Figure 5. Cladogram based on male mandible shape. This Wagner tree was computed from a matrix of Manhattan distances between population means and is outgroup rooted with a population from Burano, Tuscany (2n=40). The tree length is 28.7 and the difference between the patristic and Manhattan distances is 0.77.

It is evident that the sympatric 24 and 26 chromosome forms in Upper Valtellina[7] have been reproductively isolated for long enough for considerable morphological divergence to accrue and for most purposes could be regarded as separate species. The situation is, however, not that simple. Whilst the 24 and 26 chromosome populations, like some other Robertsonian populations of *Mus*, are reproductively isolated from one another[7] they are not

reproductively isolated from the ancestral all-acrocentric (2n = 40) forms.[3]
Consequently, reproductive isolation is not related to cladistic affinity and the problem of specific status for these populations is unresolved.

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Site attachment of a protelean parasite (Erythraeidae: *Leptus* sp.)

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Summary. *Leptus* sp. showed no site preference when they attached to an insect host. After the chelicera penetrated the host's cuticle a substance was secreted to cement the mite to its host.

The genus *Leptus* (Acarina: Erythraeidae) is a complex group of mites that are protelean parasites[1]. This cosmopolitan group of mites has a wide range of host species and various species members are parasitic on several orders of insects and other arthropods[1-3]. Several experiments on the rearing and behavior of *Leptus* spp. have provided valuable insight on the life history of this parasite[1,4,5]. This paper describes the attachment of *Leptus* sp. to its host.

Materials and methods. Specimens were fixed in 10% formalin, then cleaned in an ultrasonic cleaner. The mites were dehydrated in a graded series of ethanol and critical point dried. After mounting the specimens on SEM stubs, they were coated with carbon and gold. The mites were examined with an AMR scanning electron microscope at 20 kV.

Results and discussion. The mites were attached to several areas on 3 passalid beetles. There were 4 attached to the head, 6 on the thorax, 8 on the elytra, 6 on the legs and 4 on the ventral area of the abdomen (figs 1,3). *Leptus* sp. showed no preference for site attachment and a similar occurrence was observed in other species of *Leptus*[5].

After a host is encountered, the mites climbed onto the body and ran quickly over the surface, pausing several times to probe the host's integument. This searching activity lasted several min before an attachment site was chosen.

The long, slender chelicera are capable of penetrating heavily sclerotized cuticle such as the elytra. Once the penetration of the host cuticle is completed, the mites extended their legs toward the posterior portion of their body and the legs made no contact with the host's cuticle (figs 1,3). Then a viscous substance was secreted around the junction of the host's cuticle and the mite's mouthparts (figs 2,4). This material hardened and secured the mite to the host and then feeding was initiated.

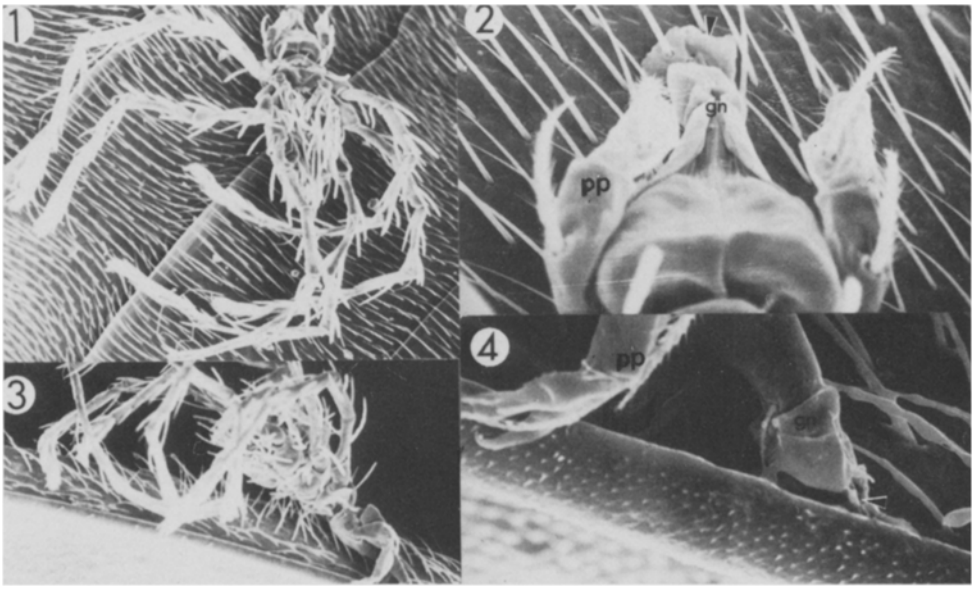

Figure 1. *Leptus* sp. attached to the venter of the abdomen. ×400. Figure 2. Higher magnification of fig. 1 showing the secreted attachment substance indicated by the arrow, gn, gnathosoma; pp, palp. ×1200. Figure 3. Larval parasite attached to the elytra. ×425. Figure 4. Higher magnification of fig. 3, the arrow indicated the attachment exudate. ×1500.

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