Duplications of additively acting genes in the evolution of a plant *(Microseris pygmaea)*

K. Bachmann, A. W. van Heusden, K. L. Chambers and H. J. Price

Hugo de Vries-Laboratorium, University of Amsterdam, Kruislaan 318, NL-1098 SM Amsterdam (Netherlands), Department of Botany, Oregon State University, Corvallis (Oregon 97331, USA), and Department of Soil and Crop Sciences, Texas A and M University, College Station (Texas 77843, USA), 2 November 1984

Summary. Crosses between *Microseris pygmaea* (10 pappus parts per fruit) and *M. bigelovii* (5 pappus parts) have revealed 10-determining genes that additively determine the average number of pappus parts in the hybrids. One or two such genes are found in different populations. Two independent duplications of the original 10-determining gene seem to have occurred.
Key words. Asteraceae; *Microseris;* speciation; quantitative genetics; polygenes; gene duplication.

Microseris pygmaea is a small dandelion-like plant of Central Chile and the only representative of its genus in South America. Near relatives (e.g. *M. bigelovii*) occur on the Pacific coast of California[1]. *M. pygmaea* differs from these in having a pappus of 10 leaf-like parts on each fruit (achene), while *M. bigelovii* has five pappus parts. In hybrids between the two species all numbers from 5 to 10 are found on achenes of the same plant[2] with an average value that is determined by the arithmetic mean of the contribution of 5- and 10-determining alleles at up to four loci[3]. The additive action of the *pappus-part* genes in these species has been analyzed in detail in two strains derived by spontaneous selfings from two hybrids between *M. pygmaea* and *M. bigelovii*[3]. In both hybrids the *M. bigelovii* parent has contributed four unlinked 5-determining genes. The *M. pygmaea* parent (from strain C30) of one hybrid (C34) has contributed a single 10-determining gene, the *pygmaea* parent (from C37) of the other hybrid (B87) two such genes. Intensive selection and linkage studies with these two hybrid strains[3] have shown that both 10-determining genes of *M. pygmaea* are allelic to 5-determining genes of *M. bigelovii*. The two (in hybrid C34: three) other 5-determining genes have no (active) alleles in *M. pygmaea*. Together with biogeographical arguments[4] these results suggest the following evolutionary history of these species: A common Californian ancestor with a single 5-determining gene has given rise through two or three gene duplications to *M. bigelovii* strains with four such genes. *M. pygmaea* is the result of chance long-distance dispersal (most likely by a migrating shore bird) to Chile[5]. Since all *M. pygmaea* have 10 pappus parts, this species derives from the establishment of a single ancestral specimen in Chile in which a '5 to 10' mutation at the original pappus part locus had become homozygous. Subsequently some populations of *M. pygmaea* have duplicated this gene.

Support for this reconstruction of the origin of *M. pygmaea* has come from the study of enzyme alleles, primarily from the gene *Got-2* coding for glutamate-oxaloacetate transaminase (E.C. 2.6.1.1)[4]. All Californian annuals of *Microseris* studied so far share a single allele at this locus. This same 'Californian' allele is found in three coastal populations of *M. pygmaea* in Chile, while all inland populations of this species share a different 'Chilean' allele of *Got-2*. Presumably the 'Californian' allele has been introduced with the ancestral specimen, and the mutation to the 'Chilean' allele and its fixation have taken place during the spread of *M. pygmaea* inland. Other enzyme genes have recently been shown to fit this pattern too[6]. This can only be expected if these alleles are either adaptively neutral or if adaptive evolution parallels the geographic spread of the species, and also if human activity has not disturbed the historical distribution pattern.

Since the additive action of the genes determining pappus part numbers has been established and the number of 10-determining genes in two strains of *M. pygmaea* has been ascertained, we can now calculate the number of 10-determining genes in various strains of *M. pygmaea* directly from the average number of pappus parts of F1 hybrids with *M. bigelovii* and check how duplications of the pappus part genes fit into our reconstruction of the evolutionary history of *M. pygmaea*. For this purpose we have crossed representative specimens of 8 Chilean populations of *M. pygmaea* (characterized in Bachmann et al.[6]) with a single tester strain of *M. bigelovii*. This is inbred strain C93 derived from a population at Pt. Sal, Santa Barbara County, California. *M. bigelovii* C93 has been selected as common tester strain since it contains more marker genes than any other strain of the species in our collection. These include two enzyme genes that are closely linked to known pappus part loci *(Esterase-1* and *Esterase-B)*[3]. The alleles at these two loci in all strains of *M. pygmaea* differ from those of the tester strain C93. Moreover, C93 contains two markers which let us detect hybrids very early in development. Since the annual species of *Microseris* self-fertilize readily, a fruiting head (capitulum) that has been cross-pollinated will usually produce two types of offspring: hybrids and maternal-type selfs[1]. Strain C93 is marked by the recessive *spatulate leaves* allele[7] and by a dominant allele for early bud development. If C93 is used as female parent in a cross, successful hybridization will be detected by the pointed leaf shape of the hybrid plantlets; if C93 is used as pollen donor, early bud development will mark hybrid plants. These identifications can be confirmed by acrylamide gel electrophoresis of the extract from a few rosette leaves. Thus the hybrid nature of a plant can be ascertained before (and independently of) the analysis of pappus part numbers.

The number of 5-determining genes in the tester strain C93 was calculated from reciprocal crosses with *M. pygmaea* C37 (the parent of hybrid B87) which contains two 10-determining genes (fig. 1). The average pappus part numbers in the 24 hybrids obtained from these crosses cluster around a value of $8\frac{1}{3}$. This is the value expected if the two 10-determining genes from C37 are balanced by a single 5-determining gene from C93. This result is very fortunate; it documents the existence of a *M. bigelovii* genome with a single 5-determining gene. Such a genome has been postulated by us as ancestral both to *M. bigelovii* strains with multiple 5-genes and to *M. pygmaea* with one or two 10-genes. Moreover, this single 5-gene will be easy to identify and map in offspring from these hybrids. This will greatly facilitate an analysis of the linkage relationships of the duplicated 10-genes.

A check of this determination using *M. pygmaea* C30 (the parent of hybrid C34) with a single 10-gene was not possible, since all 13 surviving reciprocal hybrids between C30 and C93 were small, yellowish plants that produced relatively few heads and showed extreme phenotypic instability of the pappus part phenotype.

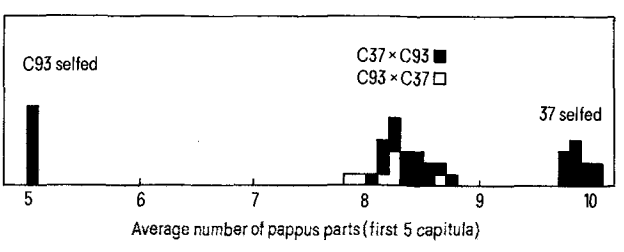

Figure 1. Distribution of the average pappus part numbers per achene in individual plants of *Microseris bigelovii* strain C93, *M. pygmaea* strain C37 and reciprocal hybrids between these strains. Strain C37 contains two 10-determining genes[3]. If C93 contains a single 5-determining gene, an average number of $8\frac{1}{3}$ pappus parts would be expected in the hybrids.

Such phenotypic instability for numerical characters[8] is common in plants and can, for instance, regularly be observed in the Ranunculaceae, where numbers of parts are not strictly canalized. Early flowers (capitula in *Microseris*) have more parts, and the number of parts steadily decreases with time and with distance from the main shoot tip. In *Microseris* instability of the pappus part phenotype is always correlated with developmental regulation. When average pappus part numbers vary among heads of one plant, peripheral achenes in each head have more pappus parts than central ones[8]. The great number of fruits on a single plant, the independent prediction of an 'expected' phenotype and the availability of genes affecting this developmental control[9] opens this effect for experimental analysis. For the present investigation, it complicates the estimate of gene numbers affecting the phenotype. Figure 2 shows the dependence of

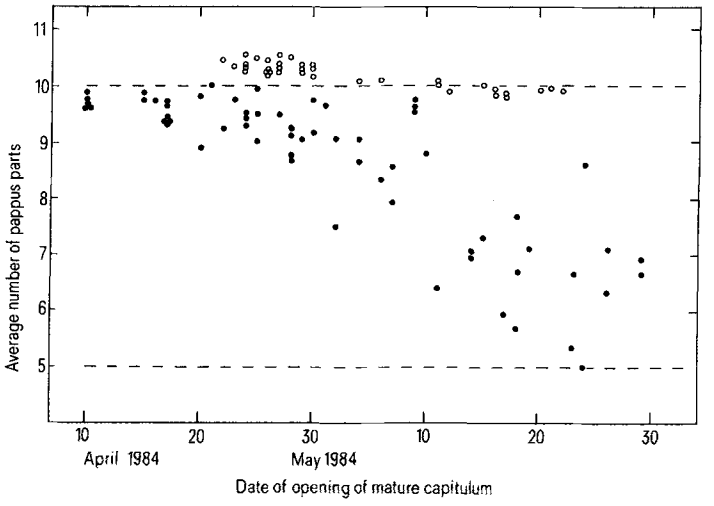

Figure 2. Dependence of the average number of pappus parts per achene in individual fruiting heads on the time of head formation. Open circles: *Microseris pygmaea* strain C93. The phenotypic instability is more marked in the hybrids, in which numerical canalization is partially broken up through the addition of a 5-determining gene. The expected phenotype of the hybrids is 7.5 pappus parts per achene. Earlier onset of maturity in the hybrids is due to a dominant gene from the pollen parent.

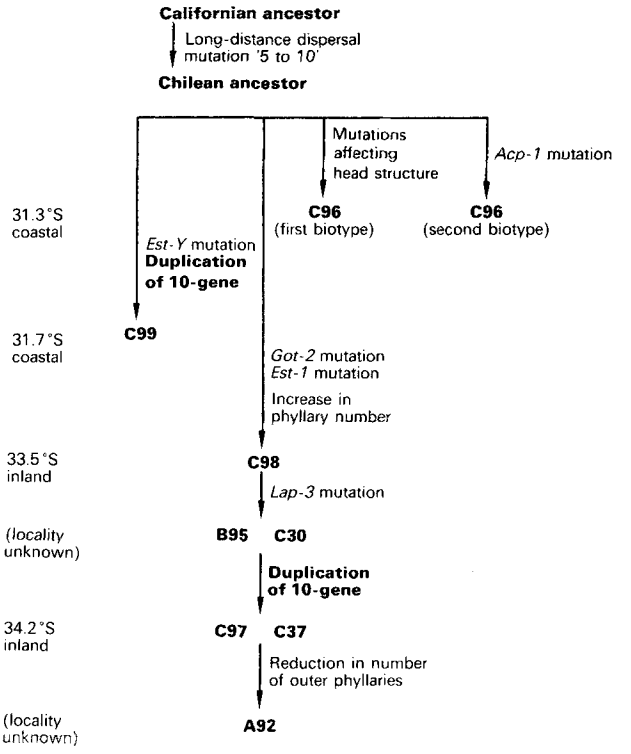

Figure 3. Tentative scheme of the evolutionary relationship of eight populations of *Microseris pygmaea* in Chile based on Bachmann et al.[6]. Mutations indicated by gene symbols refer to enzyme alleles characterized by polyacrylamide gel electrophoresis. All populations following a presumed mutation share a common allele at that locus. Data presented here on the number of 10-determining pappus part genes can be included in this scheme only if two independent duplication events are postulated.

Summary of crosses performed between eight strains of *Microseris pygmaea* and strain C93 of *M.bigelovii*, average number of pappus parts per achene determined for each combination, and the number of 10-determining pappus part genes in the *M.pygmaea* parent calculated from the average

Parental plants[a]	Number of hybrids	Number of scored achenes[b]	Average number of pappus parts	Calculated number of 10-genes	Interpretation
A92227 × C93354	3	845	7.792	1.264	2 Unstable phenotype
C93360 × A92231	1	171	8.433	2.190	
B95133 × C93360	14	5381	7.441	0.954	1
B95116 × C93336	11	4021	7.332	0.874	
C30317 × C93344	10	2571	8.725	(2.920)	1[c] Plants depauperate
C93361 × C30312	3	336	9.089	(4.490)	
C37315 × C93319	10	3091	8.365	2.059	2[c]
C37316 × C93360	7	2998	8.166	1.726	
C93333 × C37313	7	2399	8.207	1.789	
C961138 × C93355	13	4426	7.460	0.968	1
C961106 × C93352	4	1492	7.478	0.982	
C961109 × C93358	8	2961	7.707	1.181	
C97214 × C93320	6	2058	7.836	1.302	2 Unstable phenotype
C97228 × C93351	5	1941	7.737	1.210	
C93319 × C97223	2	630	8.203	1.783	
C98167 × C93332	11	1844	7.512	1.010	1
C98177 × C93320	8	1188	7.413	0.933	
C99116 × C93358	7	2543	7.635	1.114	2 Unstable phenotype
C99215 × C93355	11	3656	7.835	1.309	
C93353 × C99114	3	960	7.956	1.446	

[a] Letter and first two digits of number are strain designation; [b] based on first five mature heads; [c] number of 10-genes previously determined through breeding experiments.

the average pappus part numbers in individual heads of C30 × C93 hybrids (expected phenotype 7.5) on the date of the harvest. The same effect is seen, if on a much smaller scale, in the parental selfs obtained in these crosses (fig. 2). Obviously it is greatly magnified by the partial breakdown of numerical canalization through the addition of the 5-gene in the hybrids.

Taking into account phenotypic instability, which can be monitored by comparing the pappus part numbers in early and late capitula, the results of these crosses can be interpreted rather reliably (table). All the tested strains of *M. pygmaea* contain either one or two 10-determining pappus part genes. Additional duplication events creating four pappus part genes in the genome, as found in some strains of *M. bigelovii*, have not been detected in *M. pygmaea*. However, fitting the gene numbers found here with our scheme of the evolution of *M. pygmaea* based on enzyme gene mutations[6] requires us to assume two independent duplications of the original pappus part gene (fig. 3). One of these has led to two 10-genes in one of the coastal strains (C99), while the other must have occurred far inland, south of Santiago. This result immediately suggests further tests of our evolutionary hypothesis: All strains of *M. pygmaea* should have one 10-gene allelic to the single 5-gene from C93 (to support the notion of a single common ancestral gene). The second 10-gene in strains A92, C37 and C97 should map to another chromosome (as it does in C37) but in all three to the same (to support the common origin of the second 10-gene in these inland strains), while a different location of the second 10-gene in strain C99 would strongly support the origin of this gene by an independent duplication event.

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Circadian periodicity in termination of photorefractoriness in the yellow-throated sparrow[1]

P. D. Tewary[2] and P. M. Tripathi

Department of Zoology, Banaras Hindu University, Varanasi 221005 (India), 15 August 1984

Summary. Photorefractory male yellow-throated sparrows *(Gymnorhis xanthocollis)* were subjected to six resonance light cycles, consisting of 6 h basic photophase coupled with scotophases of longer durations (in cycles of 12 (6L:6D) h, 24 (6L:18D) h, 36 (6L:30D) h, 48 (6L:42D) h, 60 (6L:54D) h and 72 (6L:66D) h). Termination of photorefractoriness was evident in cycles of 24, 48 and 72 h, but not in 12, 36 and 60 h. The results are consistent with the 'external-coincidence' model (Bünning hypothesis) and suggest that endogenous circadian rhythmicity is involved in the termination of photorefractoriness.

Key words. Photorefractory; yellow-throated sparrow; resonance; photophase; scotophase; Bünning hypothesis; circadian rhythmicity.

Termination of the refractory period in some avian species involves a circadian component during the photoperiodic time measurement[3-8]. Investigations have, however, been limited to temperate species or to those palearctic ones which visit the Indian subcontinent or Southeast Asia. The present study was planned, therefore, to test the involvement of endogenous circadian rhythm (ECR) of photosensitivity during the termination of refractoriness in a subtropical yellow-throated sparrow *(Gymnorhis xanthocollis)*. The experimental design was similar to that developed first by Nanda and Hamner[9], now widely used as a tool for exploring circadian mechanisms. The sparrow is a resident and marked local migrant[10]. It has a seasonal reproductive cycle with a post-reproductive refractory period that needs about 8-weeks of short day (8L:16D) treatement for its dissipation (Tewary and Tripathi, unpublished data).

Materials and methods. Birds (n = 70) captured during January 1983 around Varanasi (India, lat. 25°18'N, long. 83°01'E.) were acclimatized in an outdoor aviary and moved into a short-day environment for 2 months. Such photosensitive birds were subjected to long-day conditions (15L:9D) for 120 days to make them photorefractory. During this period they were periodically examined. Completely regressed gonads (CTW = about 5 mg), followed by maximum gonadal growth (CTW = about 120 mg) indicated their photorefractory state. On 29 July 1983 such refractory birds in six groups (n = 6 each), and a control, were challenged with different programmed resonance light/dark cycles consisting of 12 (6L:6D) h, 24 (6L:18D) h, 36 (6L:30D) h, 48 (6L:42D) h, 60 (6L:54D) h and 72 (6L:66D) h for 60 days. Birds were reexamined and transferred to 15L:9D for the next 30 days to determine the dissipation of photorefractoriness. The control group was maintained under long day conditions (15L:9D) throughout experimentation. Light control was automatic (VEB Zeitschaltelektronik, Frauenstein, GDR) and also manual. Food and water were available ad libitum and were changed during the light period. All birds were lit by 20-W fluorescent tube rods, yielding an intensity of about 400 lux at cage level; the first experimental photophase was in phase with pretreatment and commenced at 06.00 h. The weight of the testes was evaluated in situ by an exploratory unilateral laparotomy as combined testicular weight (CTW), by comparison with fixed gonads of known weight. The error inherent in this method was less than 15%. Data were analyzed by Student's t-test.

Results and discussion. The data presented in the figure demonstrate that testicular recrudescence ($p < 0.001$) was evident after transfer to 15L:9D in yellow-throated sparrows which had previously been maintained under cycles of 24, 48 and 72 h (perceived as short days), but not in the control group or in those maintained under cycles of 12, 36 or 60 h (perceived as long days), despite the fact that all cycles had received only 6 h basic photophase. No significant testicular growth was evident in either the initial, finally regressed or the stimulated groups.