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Cytogenetics and karyosystematics of phyllotine rodents (Cricetidae, Sigmodontinae)

III. New data on the distribution and variability of karyomorphs of the genus *Eligmodontia*

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Abstract

Studied bone-marrow banded and Ag-NORs karyotypes of the South American phyllotine *Eligmodontia* in three populations of northern Patagonia (Argentina): south of Nahuel Huapi Lake and Los Menucos (Rio Negro Province), and Junín de los Andes (Neuquén Province). In the first locality, two polymorphic variants (2n = 34 and 2n = 32) were found; in the other two localities the 2n = 44 karyomorph previously reported as belonging to *Eligmodontia typus*, was dominant, but it was found in sympatry with two polymorphic variants (2n = 33–34) corresponding to the Nahuel Huapi karyotype, thus confirming that these karyomorphs belong to different, probably synmorphic species. The 2n = 34 variant was found for the first time in the Nahuel Huapi and in Junín de los Andes populations. G-banding and Ag-NORs proved that 2n = 44 and the polymorphic variants 2n = 32-33-34 karyomorphs are strikingly different, thus confirming a full species status for each of them.

Introduction

The desertic long-tailed phyllotine mice of the genus *Eligmodontia* are widely distributed in the southern cone of South America, from south of Perú to Tierra del Fuego. Several species have been named (according to review in Tate 1932), but after Hershkovitz's revision (1962), it was currently considered that the genus comprises only one species with two subspecies: *E. typus typus* and *E. typus puerulus* (Cabrera 1961; Honacki et al. 1982). However, in a recent paper on the karyosystematics of several Argentinian populations (Ortells et al. 1989), three different allopatric karyomorphs (2n = 44, 2n = 32-33 and 2n = 50) have been reported, stressing the view that *Eligmodontia* is a polytypic genus. The 2n = 44 form was ascribed to the type species *E. typus*, and that of 2n = 50 (previously described by Pearson and Patton 1976) to *E. typus puerulus*, whereas the polymorphic 2n = 32-33 form was of no definite identification, being assigned to *Eligmodontia* sp.

New and recent collecting in northern Patagonia allowed us to gain new knowledge on the distribution of 2n = 44 and 2n = 32-33 chromosomal species, finding that the two karyomorphs occur in sympatry, and that they are strikingly different in the Ag-NOR and in G-banding pattern. It also allowed us to find the so far unknown 2n = 34 variant of the polymorphic form, and to study the meiotic behavior of the heterozygous 2n = 33 variant.

Material and methods

Thirty-one animals from three different localities in northern Patagonia (Argentina) have been processed in this study. Two females were obtained by Oliver Pearson (University of California, Berkeley) in the steppe biome 13 km south of Nahuel Huapi Lake (Rio Negro Province, Fig. 1). Four females and nine males were caught by Allan Dickerman (University of Wisconsin) and Adrian Monjeau (University of Comahue, San Carlos de Bariloche), 15 km S.E. of Los Menucos (Rio Negro Province). Ten females and six males were collected at Estancia Quenquetrén, 110 km southeast of Junín de los Andes (Neuquén Province) by Andrés Novaro and Angel Capurro (University of Buenos Aires). Animals were captured in the field with Sherman live traps and processed in the laboratory.

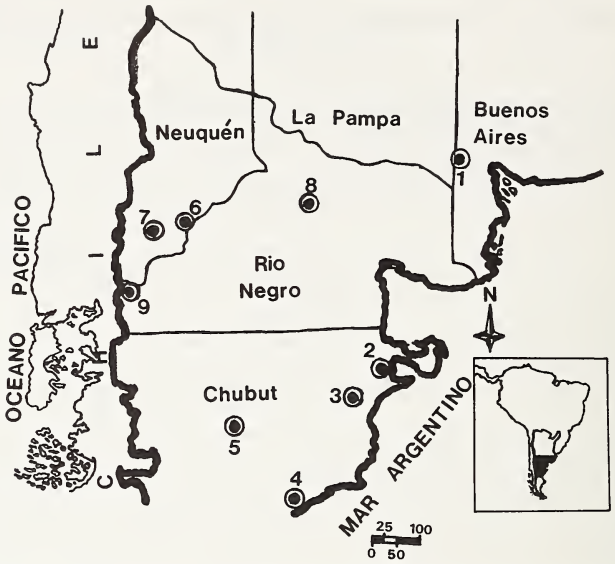

Fig. 1. Map showing where samples of *Eligmodontia* were collected and localities. 1 = Chasicó, 2 = Puerto Madryn, 3 = 28 de Julio, 4 = Pampa Salamanca, 5 = Paso de Indios, 6 = Los Lagos, 7 = Junín de los Andes, 8 = Los Menucos, 9 = Nahuel Huapi

Bone-marrow metaphases were obtained following a modified technique of Rothfels and Siminovich (1958). Testicular material was treated according to some modifications of Evans et al. (1964). Some specimens were also previously treated with yeast following Lee and Elder (1980). Ag-NOR identification was performed according to Howell and Black (1980). Buffered Giemsa stain at 10 % was used to stain mitotic and meiotic preparations, but a 3 % concentration was used in Ag-NORs staining. G- and C-banding was obtained in a few specimens following Seabright (1971) and Sumner (1972), respectively. Voucher specimens were deposited in the collection of mammals at the Municipal Museum of Natural History of Mar del Plata and the Museum of Zoology, University of Michigan. Chromosomal lengths were expressed as a percentage of the female haploid set (FHS) and calculated from a minimum of 10 metaphases. Chromosome size classes followed Reig and Kiblisky (1969), calling "large" those chromosomes > 9 FSH, "medium-sized" those between 9 and 5.5 FHS, "small" those between 5.5 and 2 FHS and "minute" those < 2 FHS. Chromosome nomenclature according to centromere position followed Levan et al. (1964). FNa values are autosomal arm numbers.

Results

Fifteen animals from Junín de los Andes and twelve from Los Menucos showed equally the 2n = 44, FNa = 44 karyotype. A male from Los Menucos presented a 2n = 33, FNa = 32 karyotype; a female from Junín de los Andes and a female from Nahuel Huapi showed a 2n = 32, FNa = 32 karyotype. A female from Junín de los Andes and another from Nahuel Huapi had an identical karyotype of 2n = 34, FNa = 32.

The 2n = 44, FNa = 44 karyomorph

Karyotype

This karyotype agrees with that previously described as belonging to *E. typus* (Ortells et al. 1989). It comprises one very large pair of metacentric (m) and twenty pairs of acrocentric (t) autosomes gradually decreasing in size. Autosomes of pair one are very large and almost fully metacentric (m); their total length is 2.5 times the length of autosomes of pair 2, and each of their arms is larger than the total size of pair 2 autosomes. The remaining autosomal pairs are distributed according to their size: those of 2 and 3 are large; those of pairs 4, 5 and 6 are medium-sized; those of pairs 7 to 20 are small, whereas those of pair 21 are minute. The XY sexual pair comprises the X chromosome, which is metacentric (m) and has the same size as autosomes of pairs 2 and 3. The Y chromosome is subtelocentric (st) and as small as the autosomes of pair 12.

Ag-NORs localization

In the 2n = 44 karyotype the Ag-NORs are located in two large and three medium-sized acrocentric autosomal pairs and in the 21 pair, the smallest autosome of the complement; this one has a low staining frequency. Although the theoretical value is twelve, in most of the plates we found from 5 to 8 chromosomes with Ag-NORs (Fig. 2a). A high frequency of association was found between two of those elements, and occasionally, between three of them (Fig. 2c).

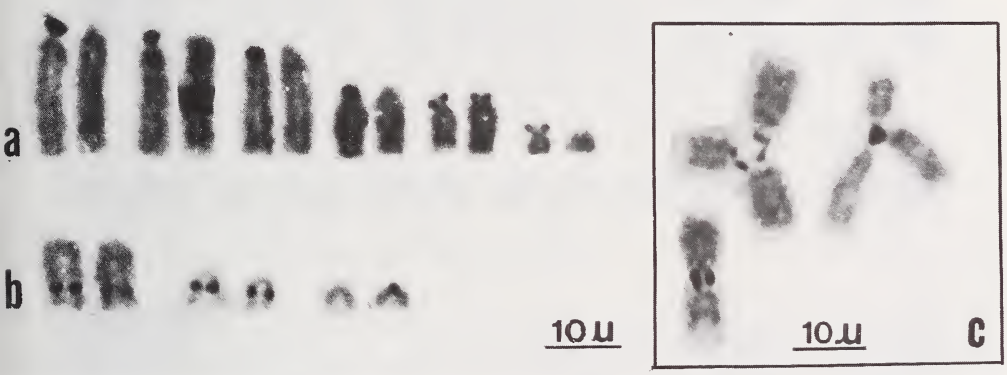

Fig. 2. a: Ag-NOR labeled 2n = 44 karyomorph chromosomes. *b:* NOR association between 2n = 44 karyomorph chromosomes. *c:* Ag-NOR labeled 2n = 33 and 34 karyomorph chromosomes

Complexes of 2n = 32-33-34, FNa = 32

Karyotype 2n = 32

An *Eligmodontia* female from Nahuel Huapi had a 2n = 32 karyotype; it agrees with that of the specimen previously studied as belonging to *Eligmodontia* sp. collected from Los Lagos (Junín de los Andes) (Ortells et al. 1989). Pairs 1 to 6 comprise large acrocentric (t) autosomes; pair 7 is made of medium-sized metacentric (m) autosomes, and pairs 8 to 15 are acrocentric (t) chromosomes, decreasing gradually in size from medium to small. The fundamental autosomal number (FNa) is 32. The X chromosomes are of medium size and acrocentric (t) similar to the autosomes of pair 8.

Karyotype 2n = 33

A male from Los Menucos showed a 2n = 33 karyotype, in which the sexual and fifteen autosomal pairs were acrocentric (t), plus an odd metacentric (m) autosome. The fundamental number is FNa = 32 (Ortells et al. 1989). Autosomes of pairs 1 to 6 are large, decreasing gradually in size; the seventh position is occupied by the odd medium-sized metacentric (m); pairs 8 to 15 decrease gradually in size from medium to small. There is an evident size gap between the first six pairs and the remaining set of autosomes. The X chromosome is medium-sized and acrocentric (t) similar to autosomes of pair 8; the Y chromosome is comparable to chromosomes of pair 14. We have been unsuccessful in obtaining good C- and G-banding in this specimen. Pairs 4 and 9 have prominent secondary constrictions located in a medial position, but in pair 4 they are closer to the telomeric end, whereas in pair 9 they are closer to the centromere.

Karyotype 2n = 34

A female from Estancia Quenquetrén and another one from Nahuel Huapi presented a 2n = 34 karyotype in which all 34 elements are acrocentric (t) keeping the FNa of 32. Comparing the 2n = 32 and 33 karyomorphs with that of 2n = 34, the latter does not present a medium-sized metacentric (m) autosome, but instead one additional small acrocentric autosomic pair (Fig. 3).

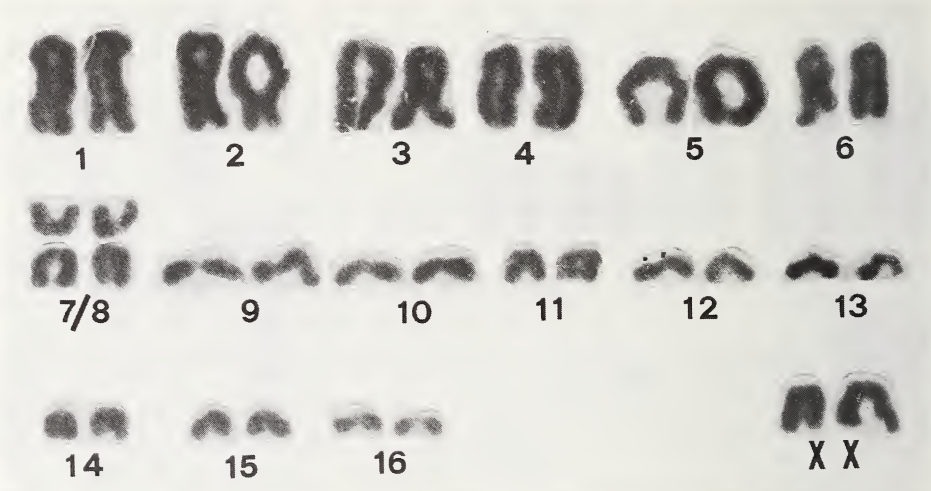

Fig. 3. Bone marrow standard Giemsa staining karyotype of *Eligmodontia* sp. from Estancia Quenquetrén (Junín de los Andes), Neuquén Province, Argentina. 2n = 34; FNa = 32

Ag-NORs localization

In the complexes of 2n = 33 and 34, Ag-NORs staining was identical. The rRNA cistrons of pairs 4 and 9 are in the same morphological position as the secondary constriction. One of the pairs from the group 12 to 15 (where all the autosomes have similar size) carries NORs, which are located in a distal pericentromeric position (Fig. 2b). Pair 9 shows the highest frequency and intensity of staining and the two homologues are usually dyed. Pair 4, which presents a lower intensity of silver staining, is usually stained in the two homologues. Silver was fixed only in a few times on the smallest pair and only one homologous showed Ag-NOR.

Meiosis

Meiosis was studied in the 2n = 33 male. In diplotene, diakinesis and metaphase I, fourteen autosomic bivalents and the XY pair with the classic end-to-end association were observed. Moreover, a characteristic autosomic trivalent was found. It was constituted by two acrocentric elements and the metacentric, which corresponds to the odd biarmed chromosome found in somatic cells (Fig. 4). Six bivalents corresponding to the first six pairs of somatic autosomes are noticeable due to their larger size. The largest pairs form two or three chiasmata. Two chiasmata are regularly shown in three large bivalents, and one or two chiasmata occur in two large bivalents. The remaining bivalents show only a bridge.

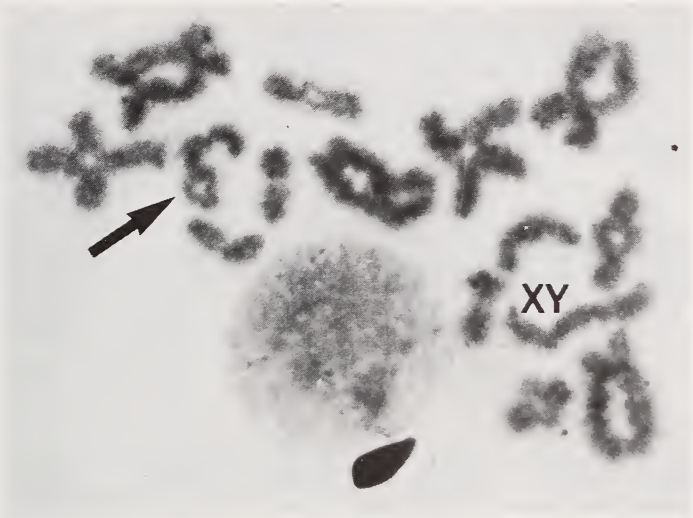

Fig. 4. Diakinesis of the male *Eligmodontia* sp. from Los Menucos, Rio Negro Province, Argentina. 2n = 33; FNa = 32. The arrow points to the trivalent

G- and C-banding

G- and C-bands were obtained in the 2n = 32 and 2n = 34 metaphases from Nahuel Huapi and compared with the 2n = 44 bands previously obtained (Ortells et al. 1989). The two karyomorphs of Nahuel Huapi fully match in arm-to-arm G-bands comparisons, allowing to confirm that metacentric pair 7 of the 2n = 32 karyotype was the result of a Robertsonian fusion of acrocentrics of the 2n = 34 karyotype (Fig. 5a, b).

C-banding of the 2n = 32 karyotype showed pericentromeric positive staining in autosomal pairs 1, 5 to 8 and 11 to 15, as well as in the sex chromosomes. C-banding of 2n = 34 specimen fully matches the pattern found in the 2n = 32 karyotype.

When comparing the 2n = 34 G-bands with those of the 2n = 44 karyotype, few arm-to-arm correspondence was found (Fig. 5c). The metacentric autosomes of the first pair of the 2n = 44 karyotype match the banding pattern of autosomes 2 and 3 of the 2n = 34 karyotype. We found an apparent lack of two bands, one pericentromeric and another telomeric in the long arm of the 2n = 44 metacentric, and another band which is present in the short arm but absent in autosomal pair 3 of the 2n = 34 karyotype. Complete homology was also found in four other autosomal pairs between the two karyotypes (Fig. 5c).

Moreover, regarding the remaining chromosomes, the 2n = 34 karyotype showed 10 autosomes and the X which are not found in the 2n = 44 karyotype, and the latter shows 16 non-shared autosomal pairs.

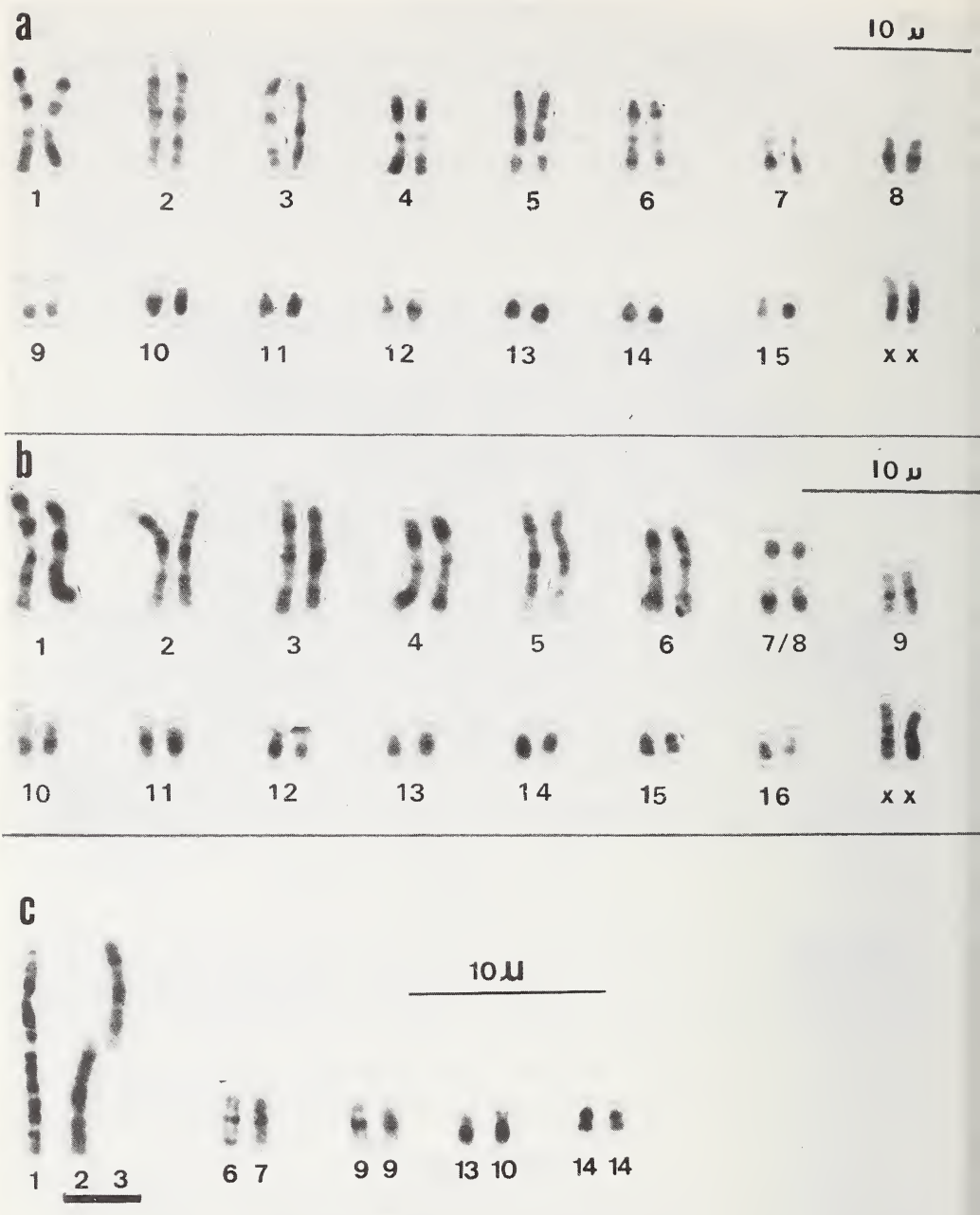

Fig. 5. G-banded karyotypes of *Eligmodontia* sp. from south of Nahuel Huapi Lake, Rio Negro Province, Argentina. *a:* 2n = 32; FNa = 32. *b:* 2n = 34; FNa = 32. *c:* G-banding pattern comparison between *Eligmodontia typus* (left) and *Eligmodontia* sp. (right). The numbers correspond to their position in each species' karyotype

Discussion

The 2n = 44 karyotype found in Los Menucos and Junín de los Andes was identical to the one previously reported from Chasicó (Buenos Aires Province) (Hurtado and Wainberg 1977) and from the same locality and central Chubut Province and referred to as *E. typus* (Ortells et al. 1989). This allows us to extend the distribution of this species 750 km to the west and 800 km to the north of previous chromosomally based records. The 2n = 43 variant found in Chasicó was not found in our new material.

The previously reported 2n = 32-33 karyomorphs from Los Lagos, south of Neuquén Province (Ortells et al. 1989) were found to occur in three new localities: in northern Rio Negro (Nahuel Huapi), south-west of Neuquén (Junín de los Andes) and north-central Rio Negro (Los Menucos), thus confirming the widespread occurrence of this form in northwestern Patagonia. The new samples also allowed to find a 2n = 34 variant of the same polymorphic system which was previously unknown. The study of meiosis of the 2n = 33 heterokaryotype and G-banding comparisons between 2n = 32 and 2n = 34 variants confirmed that all these chromosomal forms belong to one polymorphic system involving a Robertsonian fusion.

The present study also showed a frequent association between two NOR-bearing chromosomes, and an occasional one among three Ag-NOR-marked chromosomes in the 2n = 44 karyotype. This could be related with a tendency toward centric fusions. However, it is evident that it accounts for a part of the chromosomal differences between these two karyomorphs.

The placement of the Ag-NORs in different autosomal pairs and the scarce arm-to-arm homology found between the 2n = 43-44 and the 2n = 32-33-34 karyotypes confirm that a large amount of chromosomal repatterning was involved in the evolution of these two forms, bolstering the previous claim that they belong to two different, albeit probably synmorphic species (Ortells et al. 1989). This conclusion is further confirmed by the finding of sympatry of the two karyomorphs in Los Menucos and Junín de los Andes.

Thus, there is sufficient evidence to confirm that two species of *Eligmodontia* inhabit northern Patagonia. The name to apply to the 2n = 32-33-34 form is still obscure, and this problem must be solved by a thorough taxonomic revision of the entire genus.

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Zusammenfassung

Cytogenetik und Karyosystematik von phyllotinen Rodentia (Cricetidae, Sigmodontinae). III. Neue Daten über Verbreitung und Variabilität von Karyotype der Gattung Eligmodontia

Karyotypen der südamerikanischen Rodentia-Gattung *Eligmodontia* von 3 Populationen aus Nordpatagonien wurden untersucht. Tiere einer Population entstammten der Region südlich des Sees Nahuel Huapi, die einer anderen waren von Los Menucos (Provinz Rio Negro) und die der dritten von Junín de los Andes (Provinz Neuquén). In der ersten Population wurden zwei polymorphe Varianten (2n = 34 und 2n = 32) gefunden. In den beiden anderen war der bislang *Eligmodontia typus* zugeordnete Karyotyp (2n = 44) dominant. Sympatrisch zusätzlich gefunden wurden jedoch auch zwei polymorphe Varianten (2n = 33; 2n = 34), die dem Typus der Nahuel Huapi-Population glichen. Die Variante 2n = 34 wurde erstmalig für die Nahuel Huapi Region und die Population von Junín de los Andes belegt. G-Bandenmuster und vergleichende Untersuchungen am Nucleolus-Organisator nach Dar-

stellung mit Silbernitrat bestätigen, daß die Karyotypen 2n = 44 und die polymorphen Varianten 2n = 32-33-34 auffallend verschieden voneinander sind. Beiden muß sehr wahrscheinlich ein eigener Species-Status zuerkannt werden.

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