hand this notion is perhaps not so unexpected if one takes into account the fact that Melchior carried out his investigations at the Kukpuk River in the extreme north-west of Alaska. This region is separated from the rest of the *C. parryi* region by the Yukon, and since Alaska was mostly free of ice during the glacial period[7] it cannot be ruled out that in the region north of the Yukon there is a pleistocene relic population of *C. undulatus*.

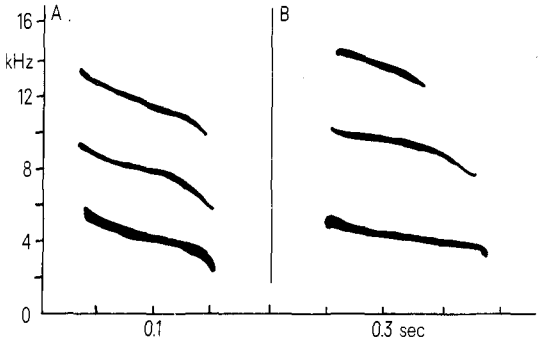


Figure 3. Whistling aerial predator alarm calls (narrow band sonagrams). *A Citellus 'parryi'*, North-west of Alaska (according to Melchior[5]); *B Citellus undulatus*, Mongolia (Khangai).

It is known that both species considered here spread into Asia from America during the pleistocene age, and that *C. undulatus* was the first to migrate, this species' location in East Siberia being in turn occupied by *C. parryi* in the late pleistocene[3]. It is therefore quite possible that *C. undulatus* survived not only in South Siberia and Central Asia but also in its native North America. The calls described here support this hypothesis; however, a final judgment must await results from further investigations. Research on the calls of squirrels living in the region south of the Yukon would be of great interest and could well decide the issue.

1 In the American literature the genus is often termed Spermophilus.
2 J.W. Robinson and R.S. Hoffmann, Syst. Zool. *24*, 79 (1975).
3 E.A. Liapunova and N.N. Vorontsov, Experientia *26*, 1033 (1970).
4 A.A. Nikolski, Zool. Zhurnal *58*, 1183 (1979).
5 H.R. Melchior, Oecologia *7*, 184 (1971).
6 Melchior uses the outdated terminology *'Citellus undulatus'* for *C. parryi* as may be inferred from the English name used for the species.
7 P. Woldstedt, Das Eiszeitalter, vol. 3. Enke, Stuttgart 1965.

Mating behavior and cytogenetical aspects of sex-inversion in the fish *Coris julis* L. (Labridae, Teleostii)

B. Duchac, F. Huber, Hj. Müller and D. Senn

Humangenetik, Departement Forschung der Universitätskliniken, Kantonsspital, CH-4031 Basel, and Zoologische Anstalt der Universität, CH-4031 Basel (Switzerland), 1 April 1981

Summary. It was observed that in the fish *Coris julis* L., in its natural environment, both primary and secondary males take part in reproduction. Chromosome studies showed 23 homologous chromosome pairs, which are identical in males and females, and a variable 24th pair. The heteromorphism of this pair is identical in secondary males and in the majority of females; these are presumably the females that can undergo sex inversion. Primary males show a different heteromorphism of the same pair.

In the order Perciformes sex inversion (from female to male) is a common phenomenon. In the species *Coris julis* L. (Labridae, Teleostii) 2 morphologically different types occur which were originally considered to be different species:

1. *Coris giofredi* Risso (with a greenish brown back and a pale yellow belly, displaying regional differences) representing the females and primary males. Of the giofredi type about 70% of the animals were found to be females and 30% primary males.
2. *Coris julis* L. (characterized by the lateral occurrence of an orange zigzag band and a blue spot), representing the secondary males.

During September and October some females turn into secondary males. This change is not only reflected in their outer appearance, but also in the morphological characteristics of the gonads as well as in the animals' behavior[2]. Environmental factors, i.e. physical and social interactions between individuals, as well as genetical factors are regarded as influencing this sex reversal[2-12]. This paper presents behavioral and cytogenetical aspects of the sex change in *Coris julis*.

Material and methods. In field observations on the coast of Elba (summer 1979 and 1980) and at Banyuls-sur-Mer (March 1980), we investigated the territorial and mating behavior of *Coris julis*. For complementary chromosomal, histological and electron-microscopical studies 70 live animals were transported to Basel in thick plastic bags filled with just enough water to cover the fish, topped up with pure oxygen before sealing.

In order to study the chromosomal aspects of sex change, fibroblastic cells were cultured according to a method described by Ahne[13]. The sex of the animals (primary males and females) was determined by studying the gonads. From cell cultures of 12 females, 5 secondary males and 3 primary males, karyotypes were made. Difficulties were

Number of animals studied with different last chromosome pair

Sex of animals	Morphological characteristics		
	2 large acrocentric chromosomes	1 large and 1 small acrocentric chromosome	1 large acrocentric and 1 metacentric chromosome
Females I	2	–	–
Females II	–	10	–
Secondary males	–	5	–
Primary males	–	–	3

encountered in obtaining a sufficient number of living cells for culturing. Kidney, swimbladder, heart and intestinal tissue were used. Dispase II (Boehringer Mannheim 1.2 units/ml in Puck's salt solution, No. 295825) was used instead of trypsin to dissolve the tissue, because it is better tolerated. The cell suspension was adjusted to a final concentration of about 2×10^6 cells/ml BME with Hanks' salts (Gibco) containing 15% calf serum (Gibco) and then cultivated in 5 ml-Leighton tubes provided with lamellae on which the cells could attach and divide. The concentration of NaCl was adjusted to 12 g/l BME. For chromosome preparations cell cultures were treated with 0.075 M KCl for 20 min and then fixed in 1:3 acetic acid alcohol[14]. The chromosome preparations were stained with 3% Giemsa (Merck, No. 9204) in phosphate buffer, pH 6.88 (Merck, No. 7294). Karyograms were made from photographs of 30 cells/animal.

Results. a) Mating behavior. From mid-July to September the mating behavior of females and secondary males could be observed. First, the female attracts the attention of the secondary male in the following manner; all fins are held closely to the body and the hind part is bent downwards (fig. 1). The secondary male appears from behind, all fins pointing away from the body except for the ventral ones (fig. 2). He swims over the back of the female touching her with his anal fin. Both animals move slowly together in spirals towards the surface of the water (fig. 3), only using their pectoral fins.

After several such spirals, the secondary male suddenly shoots upwards followed by the female. They turn back and come down again, still swimming fast. At the turning point, an abrupt rotation of the female brings its genital aperture close to that of the male. At this point both release their gametes into the water. The spawning process occurs within seconds.

As a remarkable fact we could occasionally observe the presence of a 3rd fish of the giofredi type, which always rushed to the scene at the moment of the female's rotation, in order to be close to the spawning couple; it was chased away by the secondary male after the mating act. We interpret this animal to be a primary male trying to share in the mating process. However, this cannot be proved as yet, because it is not possible to differentiate between females and primary males judging from appearance alone.

b) Cytogenetic studies. The total chromosomal number was found to be 48 in all individuals. Karyotype analyses revealed that females and secondary males have 10 metacentric and 38 acrocentric chromosomes, while primary males exhibit 11 metacentrics and 38 acrocentrics. 23 homologous pairs are identical in males and females. The remaining 2 chromosomes exist in the following 3 combinations (table): 1. 2 similar large acrocentric chromosomes; 2. 1 large acrocentric and one small acrocentric chromosome of the size of the smallest other chromosomes; 3. 1 large acrocentric and one medium-sized metacentric chromosome.

Females, primary males and secondary males have the one large acrocentric chromosome in common; this is the largest of all the chromosomes. In females only the 1st 2 combinations are present. The secondary males consistently showed combination No. 2 (fig. 4). Combination No. 3 was exclusively found in the primary males (fig. 4). In the cell cultures from 1 primary male, occasionally 49 chromosomes were counted, possibly reflecting a mutation which occurred in vitro.

Discussion. On cytogenetic grounds, 2 kinds of females could be distinguished in *Coris julis*: the minority showing 24 homologous chromosome pairs, the majority with a heteromorphous 24th pair. Our observations suggest that only the latter undergoes sex inversion because the identical karyotype was found in secondary males. It is assumed that only this type of female contains 'male' germ cells as observed in histological preparation of the gonads. Both types of females as well as primary and secondary males take part in reproduction. All produce functional germ cells. Both kinds of males were observed participating in the spawning act. Although we did not succeed in analyzing the chromosome sets of the haploid gametes, some conclu-

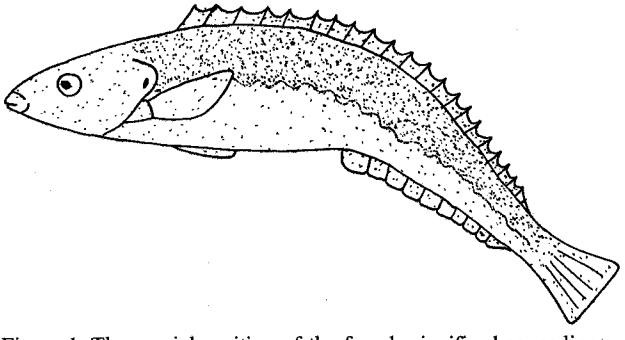


Figure 1. The special position of the female signifies her readiness to mate.

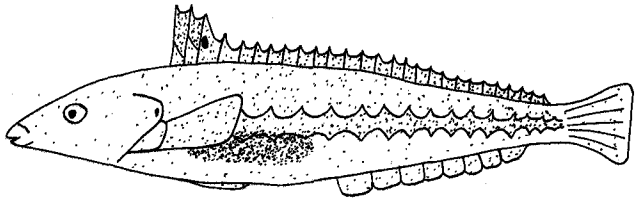


Figure 2. Mating position of secondary male.

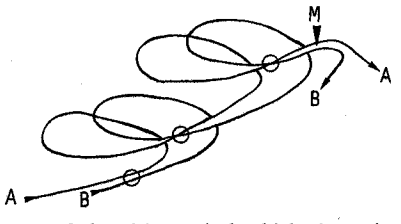


Figure 3. Diagram of the rising spiral which the pair executes during the mating process. A, secondary male; B, female; M, mating act.

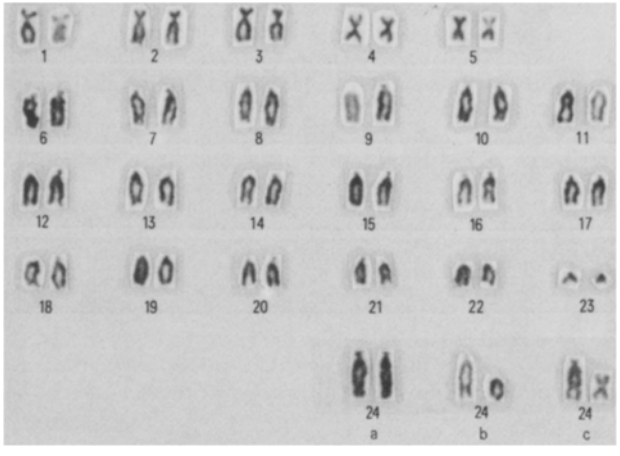


Figure 4. Karyotypes of *Coris julis*. *a*, *b* females; *b* secondary males; *c* primary males.

sions can be drawn from studies of somatic cells. A medium-sized metacentric chromosome was found exclusively in primary males, probably reflecting a male-specific chromosome. On the basis of the territorial behavior of secondary males it had been assumed that only these males reproduce. However, the persistence of this metacentric chromosome in the population proves that the conclusions drawn from observing the mating behavior were correct.

No observation of any possible offspring, with small acrocentric chromosomes, from secondary males and females could be made. Such animals could not be viable due to genetic inbalance. Through the sex inversion of some of the females into secondary males which produce functioning sperm, the ratio of females to males is substantially increased in the next generation.

A female produces relatively few but large eggs for a pelagic fish. The mechanism of sex development in *Coris julis* L. favoring the females in numbers, i.e. the female gametes, could compensate for the small number of eggs per female and allow the species to survive.

1 R. Reinboth, Zool. Anz., suppl. *24*, 259 (1961).
2 S.T.H. Chan, Phil. Trans. R. Soc. Lond. B *259*, 59 (1970).
3 S.T.H. Chan, W.S.O. and W.B. Hui, in: Intersexuality in the animal kingdom, p.201. Ed. R. Reinboth. Springer, Heidelberg 1974.
4 S.T.H. Chan, Handbook of sexology, p.91. Eds J. Money and H. Musaph. Elsevier/North-Holland Biomedical Press, Amsterdam 1977.
5 L. Fishelson, in: Intersexuality in the animal kingdom, p.284. Ed. R. Reinboth. Springer, Heidelberg 1974.
6 U. Müller and U. Wolf, Differentiation *14*, 185 (1979).
7 S. Ohno, in: The evolution of reproduction, vol.6, p.1. Eds C.R. Austin and Short. Cambridge University Press, Cambridge 1976.
8 P. Pechan, Differentiation *14*, 189 (1979).
9 R. Reinboth, Gen. comp. Endocr. *2*, 629 (1962).
10 R. Reinboth, Biol. Reprod. *22*, 49 (1980).
11 D.R. Robertson, Science *177*, 1007 (1972).
12 M.J. Roede, Vie Milieu A *17*, 1317 (1966).
13 W. Ahne and P.A. Bachmann, Zentbl. Bakt. ParasitKde I *228*, 447 (1974).
14 H.P. Klinger, Cytogenetics *11*, 424 (1972).

Hyperglycemic activity of crab and scorpion hormones in grasshopper *(Poecilocerus pictus)*[1]

P. Sreenivasula Reddy[1], A. Bhagyalakshmi, R. Ramamurthi and V. Chandrasekharam

Department of Zoology, Sri Venkateswara University, Tirupati-517502 (India), 24 November 1981

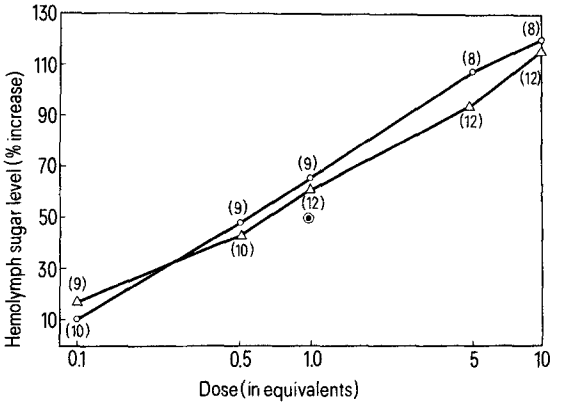
Summary. Hyperglycemic hormones obtained from crab and scorpion both cause significant, dose-dependent elevations of hemolymph sugars in the grasshopper *Poecilocerus pictus*. These results suggest a highly conservative evolution of some mandibulate arthropod neuro-hormones.

Since its discovery by Steele[2] in cockroaches, the hyperglycemic hormone has been throughly investigated in several orders[3-6]. We have shown that hemolymph sugar in the grasshopper *Poecilocerus* is also under the control of a hyperglycemic hormone, which is present in the brain[7]. Earlier research indicated that crustacean eyestalk hormones show glycogenolytic effect in grasshopper[8]. In view of these developments, we decided to examine the effect of hyperglycemic hormones of the crab[9] or the scorpion[10] on the grasshopper. Our results demonstrate that low dosages of hyperglycemic hormones obtained from crab and scorpion are effective in grasshopper and provide strong evidence for a close similarity of crab and scorpion hyperglycemic hormones and a putative grasshopper hyperglycemic hormone.

Materials and methods. The grasshoppers, *Poecilocerus pictus* used in these studies were collected in the wild in Tirupati from late August to mid October, 1980. They were fed *Calotropis* leaves at room temperature (32 °C) every day; feeding was discontinued 24 h prior to use in experiments. Crab, *Oziotelphusa senex senex* hyperglycemic hormone was extracted from eyestalks, and that of the scorpion *Heterometrus fulvipes* from the cephalo-thoracic ganglionic mass (CTGM). The hormones were solubilized and injected, and assays were conducted as previously described for grasshoppers[8]. Hemolymph sugar level was determined using anthrone reagent[11].

Results and discussion. The figure summarizes the effects of hyperglycemic hormones obtained from crab and scorpion in grasshopper. A linear dose-response relationship, at low doses, is evident for both hormones in grasshopper. For comparison, extracts of grasshopper brains, injected at a dose of 1 brain equivalent/animal raised hemolymph sugars in 2 h to 12.04 ± 1.02 µg/ml from 6.09 ± 0.84 µg/ml in 10 grasshoppers (data are mean ± SD). By contrast, distilled water injections resulted in an insignificant change of −1.3 ± 1.2 µg/ml (n = 9).

The above data demonstrate for the 1st time that 2 similar neurosecretory peptide hormones, isolated from arthropods as distantly related as decapods and arachnids, mimic the effect of a putative neurosecretory hormone[7] of grasshopper. The presence of materials with hyperglycemic hormone-like biological activity in locusts[3], stick insects[4], bees[5]



Effect of crab (○) and scorpion (△) hyperglycemic hormones on hemolymph sugar levels in grasshopper *(Poecilocerus pictus)*. Hormones (crab: eyestalks equivalents; scorpion: CTGM equivalents) injected in 10 µl distilled water; n in parentheses; sugars measured 2 h after injection. Dotted circle (⊙) is 'reference standard' indicating the elevation of glycemia caused by grasshopper brain in its own milieu (for detailed values, see text).