tested allozymic variation, as detectable by starch gel electrophoresis, of 11 classes of enzymes and other proteins, controlled by 22 genes (table 1). Plasma and erythrocyte fractions of blood were used, as well as homogenates of 4 different organs.

4 loci were found to be polymorphic (table 2). Excluding 2 of the monomorphic loci because of insufficient sampling (see below), the average heterozygosity for the sample is 0.052. This falls very close to the 0.047 average found for the lacertilians already examined at 14 loci or more[1]. The lacertilian average is in turn similar to the vertebrate average of 0.049[1].

The amount of polymorphism is found to be 20% when using the 0.01 criterion, but since observed electromorph frequencies cannot be lower than $1/(2\times 16)\simeq 0.03$ with our sample, this is more likely to be a conservative rather than an excessive estimate (An excessive estimate would occur if one of the electromorphs recorded once in our sample happened to be an exceptional allozyme in the population). With the 0.95 criterion, the rate of polymorphism is 15%, a value that seems average among vertebrates[6].

In conclusion, the genetic variability electrophoretically detected in the gekkonid population sampled does not reflect the high gene flow and active diversifying selection suggested by the ecological versatility of *Hemidactylus brooki*. The loci tested in our sample show the low variability typical of vertebrate species. Actually, the absence of variation at loci observed in 8 (*Ldh-C*, only visible in mature males) or 9 geckos (*Idh-2*, controlling a fast denaturating enzyme) suggests that variability may be somewhat lower than estimated[7].

Table 2. Electromorph frequencies at polymorphic loci of the *Hemidactylus brooki* sample of Gabon

Loci	Observed alleles	Frequencies
Idh-1	*1.00*	0.97
	1.40	0.03
a-Gpd	*1.00*	0.67
	1.35	0.33
Est-1	*1.00*	0.80
	1.19	0.20
Est-4	*0.92*	0.04
	1.00	0.88
	1.08	0.08

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7 Technical assistance: Josette Catalan.

Additional chromosome duplication in female meiotic prophase of *Sipyloidea sipylus* Westwood (Insecta, Phasmida), and its absence in male meiosis

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Summary. In the parthenogenetic stick insect *Sipyloidea sipylus*, an additional chromosome duplication takes place in the primary oocytes immediately after pachytene. After duplication, the chromosomes again appear in a pachytene configuration, and oogenesis proceeds with twice the somatic number of chromosomes. Additional duplication does not occur during prophase of primary spermatocytes.

The stick insect *Sipyloidea sipylus* Westwood reproduces by thelytokous parthenogenesis[1]. In first meiotic metaphase, the number of elements equals the somatic number of chromosomes ($2n=80\pm2$). These elements are tetrapartite and consist of 4 chromatids. The 2 meiotic divisions are normal and the somatic number of chromatids enters the pronucleus. The meiosis thus evolves with twice the somatic number of chromosomes[2]. However, it could not be established when and how the chromosomes were doubled. The meiosis of the thelytokous stick insect *Carausius morosus* Br. is rather similar to that of *S. sipylus*[3,4]. With the aid of cytophotometry it could be shown that in the former phasmid the chromosomes are duplicated after pachytene during a stage called pachyreduplication phase[5]. After doubling, they enter anew into a pachytene called tetrapachytene. Moreover it was found that in a (rare) male complete as well as incomplete duplication occurred during zygotene[6]. In order to trace the additional chromosome doubling in *S. sipylus*, cytological and cytophotometrical investigations were carried out on 2 mature ovaries and on a pair of ovotestes.

S. sipylus was bred on leaves of bramble under conditions as described for *C. morosus*[5]. The stock originated from a sample sent by Dr H.K. Van den Bergh (Antwerp) in 1976[1]. An adult male (length 60.5 mm) was obtained from the progeny of females irradiated with 500R of X-rays (11 R/sec). Its morphology conformed to the description given by Urvoy[7]. The 3rd-6th abdominal segments contained a pair of ovotestes: a tube-shaped left testis, with a narrow part at one-third from the distal end from which 2 ovarioles protruded, and a tube-shaped right testis with a narrower distal half from which 7 ovarioles protruded. The ovarioles had a normal morphology (growing oocytes up to 1 mm long). The investigations were carried out on Feulgen stained squash preparations of ovarioles and ovotestes. The slides were made as described before[5,6] (25 min hydrolysis in 5 N HCl at room temp.). The relative quantity of DNA in the nuclei was determined with a Zeiss Scanning Mikroskop-Photometer (Ø diaphragm plug 0.6 µm) at 558 nm.

In the ovary, the end chamber of an ovariole contains oogonia and oocytes in early meiosis[2]. The oocytes are in leptotene, zygotene or pachytene. A bouquet, as developed

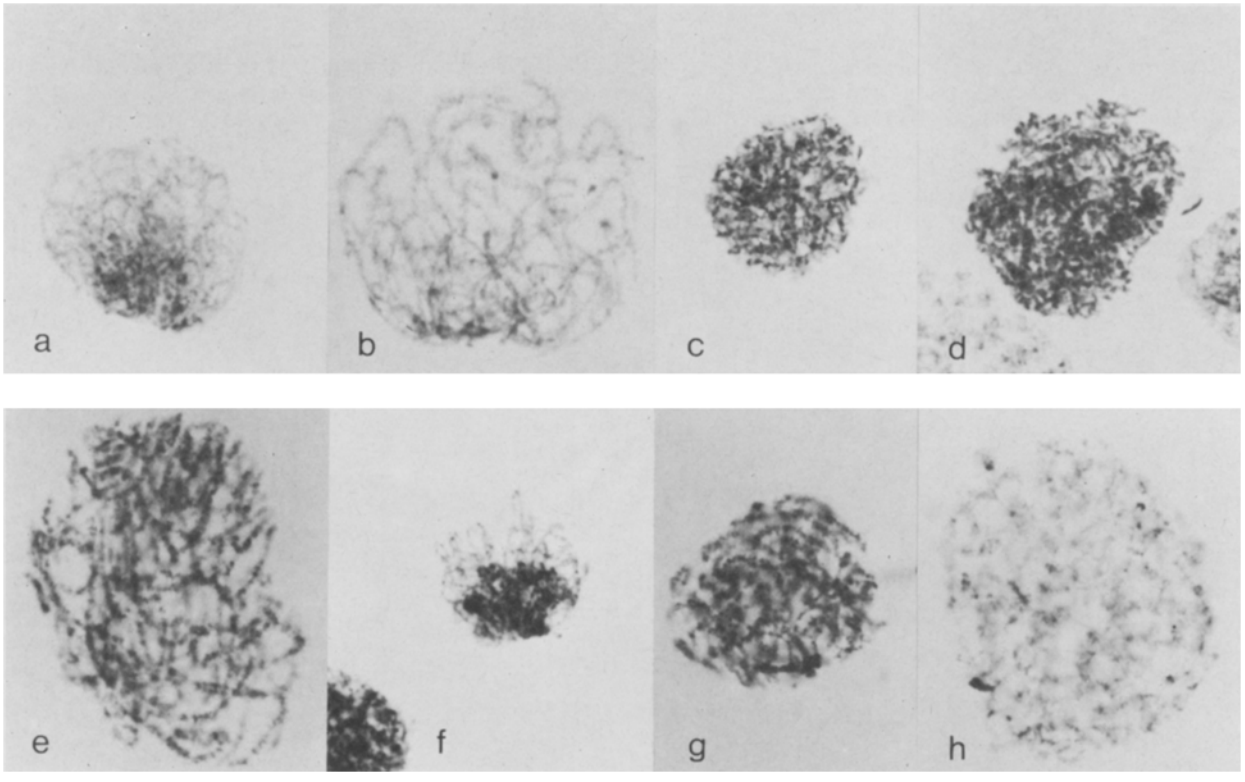

Fig. 1. Primary oocytes and spermatocytes of *Sipyloidea sipylus*. Magnification × 1000. *a–e* Oocytes: *a* zygotene/bouquet, *b* pachytene with slight heteropycnosis of some bivalents, *c* early pachyreduplication phase, *d* late pachyreduplication phase, *e* tetrapachytene. *f–h* Spermatocytes: *f* zygotene/bouquet with 2 heterochromosomes, *g* pachytene with 2 heterochromosomes, *h* diffuse stage with 2 fused heterochromosomes.

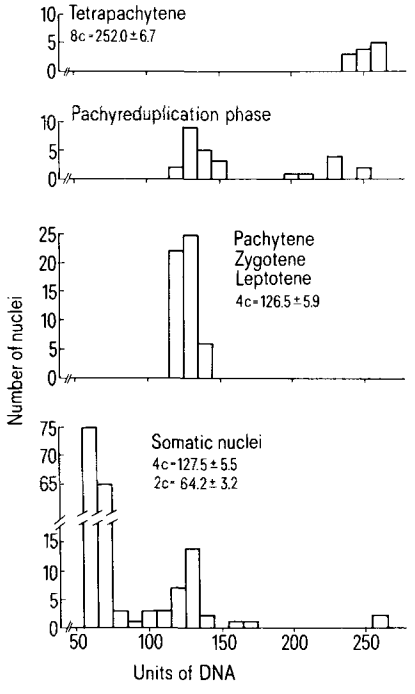

Fig. 2. Diagram of the cytophotometric DNA measurements on somatic and germ cell nuclei of ovarioles of adult *Sipyloidea sipylus*. The DNA contents are given in arbitrary units, corresponding to the integrals of absorbance. Each column on the ordinate represents 10 DNA units. The mean DNA values and corresponding c levels have been given (2c = DNA content of somatic nucleus in G1 phase).

during zygotene (figure 1, a), is less distinct during pachytene. At least 10 pairing configurations show slight positive heteropycnosis (figure 1, b). Compared with somatic nuclei, these oocytes contain the expected 4c value of DNA (figure 2). According to the cytophotometric measurements, pachytene is followed by a stage in which the DNA is replicated (4c → 8c). During this pachyreduplication phase, pairing of chromosomes is no longer visible, the chromatin appears as interphase-like threads of variable thickness (figure 1, c and d). After duplication, the chromosomes reappear as pachytene configurations in the stage called tetrapachytene (8c) (figure 1, e). The tetrapachytene bivalents do not show differences in internal coiling. Their estimated number is at least 65, which is fairly close to the somatic number of chromosomes. Then the nuclei rapidly enter into the diffuse stage of the growing oocyte.

Spermatogenesis takes place in the normal-sized parts of the ovotestes. The spermatogonial interphases contain 2 small, bent, apparently V-shaped heterochromosomes which can hardly be distinguished among the rather granular chromatin. Only few gonia were in mitosis, so that the karyotype could not be established. During first meiotic prophase, the 2 heterochromosomes have, generally, fused to various forms (figure 1, f–h). A bouquet, as present during zygotene (figure 1, f), disentangles during pachytene (figure 1, g). The autosomal bivalents do not exhibit differences in internal coiling. At the end of pachytene, the nucleus doubles its volume, which goes together with a spreading and an extension of the bivalents. The resulting diffuse stage (figure 1, h) lasts relatively long. Unlike *C. morosus*, additional DNA synthesis resulting in either complete or incomplete duplication of chromosomes was

not found in leptotene, zygotene, pachytene and diffuse stage. Diplotene and diakinesis are passed quickly. Thereafter the meiotic divisions are highly abnormal like in *C. morosus*[3,8].

Oogenesis in the end chambers of the ovarioles of the ovotestes proceeds with the same morphological stages observed in the end chambers of ovaries. Thus, heterochromosomes are not present, a slight heteropycnosis of some pachytene bivalents is evident, and an additional duplication of chromosomes takes place after pachytene.

These results show that in oocytes the chromosomes are duplicated immediately after pachytene, like in *C. morosus*, and thus give the 2nd example of this peculiar doubling system. The additional duplication makes possible the parthenogenetic reproduction. In the ovotestes, the female germ cells are morphologically similar to those in ovarioles of ovaries, and the male germ cells show the same phenotypic features as those of other stick insect species[3]. This situation is consistent with the current opinion that the phenotypic sex of the germ line in insects is determined by its chromosome constitution[9,10], admitting that our exceptional male obtained through irradiation probably was mosaic in origin.

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Glutathione peroxidase in dried blood spots

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Summary. A new procedure utilizing dried blood spots was developed for detecting glutathione peroxidase deficiency. Samples from a known patient with a partial defect and from rats with an induced deficiency were distinguished from respective control groups by their longer defluorescence endpoints. Samples from 100 patients with anemia and 2 phenylketonuric infants on low-protein diets contained glutathione peroxidase activity similar to that in 82 controls, when screened for the enzyme defect by the new procedure.

We describe a screening method for glutathione peroxidase, an enzyme, which, in vitro, catalyzes the detoxification of peroxides via glutathione in the hexose monophosphate pathway. The techniques were adapted from Beutler's fluorescent tests for other red cell enzymes[1], modified to the kinetics of peroxidase[2] and applied to the dried blood spot sample. The test should facilitate the identification of patients with peroxidase defects, only a few of whom have been described[3-6]. In our test, peroxidase activity (GSH-Px) is indicated by defluorescence, as reduced nicotinamide adenine dinucleotide phosphate (NADPH) is oxidized to NADP in a linked reaction with glutathione (2GSH < − > GSSG).

$$2\,GSH + H_2O_2 \xrightarrow{GSH\text{-}Px} H_2O + GSSG \xrightarrow{GSSG\text{-}R} 2\,GSH$$

$$NADPH \rightarrow NADP$$

Material and methods. Defluorescence times of dried blood spot samples from I.S., a patient with a partial deficiency of glutathione peroxidase[5] (personal communication from Professor Rodolfo Bracci) were compared with those of mailed spots from 40 newborns being screened for inborn errors in a state-wide program and from 100 patients with unexplained anemia, varying in age from infants to adults, and with those of fresh spots from 11 healthy laboratory workers, aged 23–55, and 34 adults undergoing blood tests as outpatients for a variety of illnesses.

The samples were obtained by saturating filter paper cards (3MM) with spots of blood and air-drying for at least 1 h. In the procedure, discs of 5 mm diameter were punched from the cards, guided into the wells of a plastic tray and overlaid with 0.2 ml reaction mixture. The solutions were mixed well with plugged hematocrit tubes. The reaction mixture contained the following ingredients prepared ahead of time: 1 ml 1 M potassium phosphate buffer, pH 7.0, 0.2 ml 0.2 M ethylenediaminetetraacetic acid, 0.1 ml 0.4 M sodium azide, 0.2 ml ferricyanide-cyanide solution (30 mg $K_3Fe(CN)_6$ + 10 mg NaCN/100 ml water) and 1.4 ml demineralized water were combined and stored for several days at −20 °C before use. The following ingredients, however, were prepared fresh and added to the solution above just before use: 0.1 ml 0.1 M glutathione (GSH), 1.0 ml glutathione reductase (10 U/ml Sigma), and 1.0 ml 2 mM nicotinamide adenine dinucleotide phosphate, reduced (NADPH). 25 λ H_2O_2 (5 mM) were added to the reaction mixtures in the wells and mixed. Aliquots of the incubation mixture were applied as single drops to a premarked Whatman No. 1 filter paper sheet immediately, mixed with a plugged microhematocrit tube and air-dried for at least 30 min. Incubation mixtures were incubated at 37 °C for at least 10 min and aliquots removed, spotted at 3-min intervals and air dried. Defluorescence was observed in a long-wave UV view box. Incubation mixture without a blood spot sample served as the positive (enzyme-deficient) control.

The accuracy of the method was ascertained by comparing defluorescence times in the blood spot samples with enzyme activities in the whole blood samples from 13 of the adult donors undergoing blood tests from whom the spot

Defluorescence times and activities of glutathione peroxidase in 13 whole blood and spot samples from adult outpatients

Defluorescence time (min)	Peroxidase activities (IU/g Hb)
3	6, 7, 7, 8, 11, 7
6	3, 4, 5, 5, 6, 6, 6, 5
9	3, 3