possibly even for human intelligence[12] suggests these may be fitness characters with high performance conferring some advantage. It would be tempting to justify the present learning task in the same way, the sequence of turns required for learning being very similar to an insect's path towards a food odour[13]. However, the main points are that directional dominance has been found in *Drosophila*, paralleling that in rodents and that it distinguishes learning from the other behavioural components in the maze. It is reassuring that similar directional dominance has recently been observed in different strains of *D. melangogaster* with a very different learning task[2] involving electric shock[14].

1 D. Menne and H.-Ch. Spatz, J. comp. Physiol. *114*, 301 (1977).
2 Y. Dudai, J. comp. Physiol. *114*, 69 (1977).
3 D.A. Hay, Nature *257*, 44 (1975).
4 W.G. Quinn and Y. Dudai, Nature *262*, 576 (1976).
5 A. Pruzan, P.B. Applewhite and M.J. Bucci, Pharmac. Biochem. Behav. *6*, 355 (1977).
6 D. Wahlsten, Behav. Biol. *7*, 143 (1976).
7 G. Bicker and H.-Ch. Spatz, Nature *260*, 371 (1976).
8 D.A. Hay, Nature *260*, 271 (1976).
9 D.A. Hay, Experientia *28*, 922 (1972).
10 K. Mather and J.L. Jinks, Biometrical Genetics. Chapman Hall, London 1971.
11 D.A. Hay and S.A. Crossley, Behav. Genet. *7*, 389 (1977).
12 J.L. Jinks and L.J. Eaves, Nature *248*, 287 (1974).
13 J.S. Kennedy and D. Marsh, Science *184*, 999 (1974).
14 D. Fulker, personal communication.

Y chromosome in the sibling species *Anopheles atroparvus* (van Thiel, 1927) and *A. labranchiae* (Falleroni, 1926) (Diptera: Culicidae): differential behaviour of the short arm after acid-alkaline treatment and Coriphosphine-O staining

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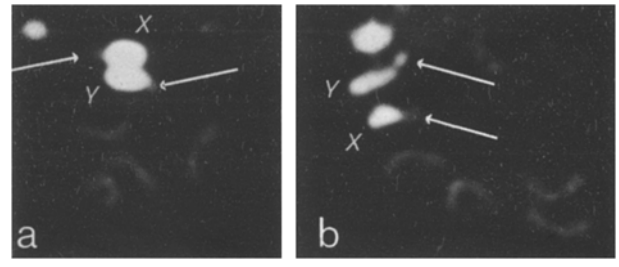
Summary. An acid-alkaline treatment followed by Coriphosphine-O staining was used for detecting chromosomal differences between the 2 sibling species *Anopheles atroparvus* (van Thiel) and *A. labranchiae* (Falleroni) (Diptera: Culicidae). The short arm of the Y chromosome was found to stain differently in the 2 species.

The paleartic *Anopheles maculipennis* complex includes 6 species of which 2, *Anopheles atroparvus* and *A. labranchiae*, are considered the most closely related genetically[2,3]. All these species show an identical karyotype (2n = 6) which consists of 2 pairs of metacentric autosomes and 1 pair of submetacentric sex chromosomes. Ethological and ecological, in addition to cytogenetical and biochemical, investigations were carried out for distinguishing the above-mentioned 6 species[4–7]. The hybridological approach, moreover, either without or in connection with the observation of polytene banding patterns, has been used for detecting specific differences, particularly between *A. atroparvus* and *A. labranchiae*[5,8,9].

The only cytological differences between these 2 species were found with Q-banding or with the labelling of replicating DNA in the hybrid polytene chromosomes[10,11]. Such differences, however, involve the whole chromosomal complement on the one hand (Q-banding), or asynaptic polytene regions on both heterosomes and autosomes on the other hand (differential DNA synthesis). The Y chromosome was never found to show specific differences between the 2 species considered, which, moreover, show complete homology in karyotype[10], DNA replication in metaphase chromosomes (Fraccaro et al.[12], and Marchi, unpublished personal observations), and polytene banding pattern[13].

Cytotaxonomic markers are now available, which can complement the banding pattern of polytene chromosomes. A number of techniques make possible a linear differentiation of metaphase chromosomes in several organisms, including Diptera[12,14,15]. We used an acid-alkaline treatment followed by Coriphosphine-O staining for differentiating metaphase chromosomes of *A. atroparvus* and *A. labranchiae*. The possible correlations between these cytological results and previous hybridological and genetical data will be discussed.

Materials and methods. A. atroparvus larvae were provided by the Genetics Institute of this University, through the courtesy of Prof. G. Frizzi, while *A. labranchiae* were collected in the field from several areas of Sardinia. Brains and/or gonads of 4th instar larvae of both sexes were dissected in a modified Ringer's solution (NaCl 0.9 g, KCl 0.42 g, $CaCl_2$ 0.25 g in 1000 ml of distilled water). After fixation in methanol: acetic acid (3:1), tissues were incubated in 45% acetic acid for 2 min. After squashing, slides were kept in solid CO_2 for 5 min and the siliconized coverslips were removed. Air-dried preparations were left to age for 5–7 days at 21 ∓ 2 °C and then processed as follows: a) immersion in 1 N HCl at 21 ∓ 2 °C for 10 min and rinsing in distilled water; b) bath in 5% $Ba(OH)_2 \cdot 8\ H_2O$ at 52 °C for 4 min and washing in distilled water; c) staining with Coriphosphine-O according to Keeble and Jay[16]. Control slides were stained with the same fluorochrome, either



Metaphase chromosomes of *A. atroparvus* (a) and *A. labranchiae* (b) after acid-alkaline treatment and Coriphosphine-O staining. *a A. atroparvus:* the arrows indicate the short arms of the sex chromosomes. The long arms reveal close pairing and bright fluorescence. The 2 autosome pairs are located under X and Y and show low fluorescent intensity like the short arms of sex chromosomes. *b A. labranchiae:* the short arms reveal a different fluorescent staining in sex chromosomes, being bright on the Y and dull like the autosomes on the X (see arrows).

without any post-fixation treatment or after immersion in 4% formaldehyde at 52 °C after acid-alkaline steps.

Results and discussion. Coriphosphine-O staining shows a red-orange colour on all autosomes in both species. The X chromosome also does not reveal differences between the species, the short arm showing a red-orange colour like the autosomes and the long arm the following pattern: the proximal and distal areas are red-orange, the interstitial region being bright yellow-green. The long arm of the Y chromosome reveals the same staining reaction in the 2 species and appears to be identical to the homologous portion of the X, while the short arm is grey-orange in *A. atroparvus* and bright yellow-green in *A. labranchiae*. This portion (figure), reveals a clearcut difference between the species.

Coriphosphine-O is a metachromatic dye already employed as stain for nucleic acids in fixed preparations[17,18]. A red-orange fluorescence is emitted when the fluorochrome is bound to RNA and a yellow-green fluorescence when it is bound to native, double-stranded DNA. Treatments producing denaturation and/or depolymerization of DNA also induce a red-orange fluorescence when the fluorochrome is bound to such a nucleic acid[19]. On the other hand, formaldehyde is known both to prevent reassociation of single polynucleotide chains after heat or high-pH denaturation, and to stabilize deoxyribonucleoproteins[20,21].

Control experiments show all chromosomal DNA in its native state (green colour) in untreated preparations, while the use of formaldehyde after the alkaline step shows denaturation of all DNA (red colour). We therefore assume that DNA strandedness is the fundamental factor causing the differential staining. Our results, then, show that in the short arm of the Y resides the only chromosomal difference demonstrable with a technique whose different staining reactions could reveal specific types of DNA or at least a particular DNA/protein packing. This cytological difference is particularly interesting when compared to the data of Jayakar et al.[22], who proposed an X-Y incompatibility mechanism as the genetical basis of sterility of the F_1 hybrid males which have an X chromosome of *A. atroparvus* and a Y chromosome of *A. labranchiae* or vice-versa. Fraccaro et al.[9], moreover, state that '... behavioural polymorphism stenogamy-eurygamy is obviously controlled by one or more genes located on the Y chromosome'.

We cannot say whether our results indicate a differential spiralization and heterochromatinization in the short arm of the Y chromosome between the 2 sibling species, given that the correlations between banding techniques and heterochromatin structure are intricate. Nor can we affirm that genetic differences between *A. atroparvus* and *A. labranchiae* are located in the short arm of the Y chromosome, although we believe that these results support the involvement of this chromosome in the differentiation of these 2 species.

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2 U. Bianchi, Riv. Parasit. *29*, 221 (1968).
3 J.B. Kitzmiller, Adv. Genet. *18*, 315 (1976).
4 G. Frizzi, Scientia genet. *3*, 67 (1947).
5 J.B. Kitzmiller, G. Frizzi and R.H. Baker, in: Genetics of Insect Vectors of Disease, p. 151. Ed. J.W. Wright and R. Pal. Elsevier, London 1967.
6 U. Bianchi, Nature Lond. *217*, 382 (1968).
7 M. Coluzzi, Misc. Publs ent. Soc. Am. *7*, 63 (1970).
8 A. Corradetti, Riv. Malar. *13*, 707 (1934).
9 M. Fraccaro, L. Tiepolo, U. Laudani, A. Marchi and S.D. Jayakar, Nature, Lond. *265*, 326 (1977).
10 R. Mezzanotte and L. Ferrucci, It. J. Zool., in press.
11 L. Tiepolo, G. Diaz and U. Laudani, Chromosoma *45*, 81 (1974).
12 M. Fraccaro, U. Laudani, A. Marchi and L. Tiepolo, Chromosoma *55*, 27 (1976).
13 A. Farci, U. Laudani and A.R. Lecis, Z. zool. Syst. Evolut.-Forsch *II*, 304 (1973).
14 F.E. Arrighi and T.C. Hsu, Cytogenetics *10*, 81 (1971).
15 S. Pimpinelli, G. Santini and M. Gatti, Chromosoma *57*, 377 (1976).
16 S.A. Keeble and R.F. Jay, Nature, Lond. *193*, 695 (1962).
17 D. Nash and W. Plaut, J. Cell Biol. *27*, 682 (1966).
18 J.E. Mayfield and J.R. Ellison, Chromosoma *52*, 37 (1975).
19 F.H. Kasten, in: Encycl. of Microscopy and Microtechnique, p. 5. Ed. P. Gray. Van Nostrand, New York 1973.
20 C.A. Thomas, Jr, and K.J. Berns, J. molec. Biol. *4*, 309 (1962).
21 R. Rigler, D. Killander, L. Bolund and N.R. Ringertz, Exp. Cell Res. *55*, 215 (1969).
22 S.D. Jayakar, U. Laudani, A. Marchi, L. Tiepolo and M. Fraccaro, J. Genet. *63*, 1 (1977).

Regeneration of plants from mesophyll protoplasts of *Atropa belladonna*[1]

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Summary. Isolated leaf mesophyll protoplasts of *Atropa belladonna* when cultured in defined liquid culture media regenerate cell walls, divide and form calli. Subsequent induction of shoot and root organogenesis leads to plantlets which grow to maturity after transfer to soil.

Isolated plant protoplasts with high viability and regeneration capacity (totipotency) are a prerequisite for genetic modification studies. Further progress in somatic hybridization between incompatible non-related species is likely in the near future within the family of Solanaceae, since protoplasts of various members of this group can already be regenerated to plants. Plantlet formation from *Atropa belladonna* cell culture-derived protoplasts has also been described[2]. However, because of desired visible markers for protoplast fusion studies and possible genetic instability of long-term cell cultures[3], we have concentrated our efforts on mesophyll protoplasts.

In vitro shoot cultures[4] were established from sterile seedlings and were subcultured at 4 weeks intervals on MS medium[5] supplemented with 0.5 mg/l BAP[6] and maintained at 25 °C, 12 h 3000 lux. 2–3 weeks after subculture of the shoots, excised leaves were cut into 0.5 mm strips and incubated for 2.5 h at 27 °C in enzyme mixture (0.5% macerozyme R 10 + 0.5% cellulase R 10 + 0.2% hemicellulase) dissolved in osmoticum (0.4 M mannitol + 5 mM