stocks does not lead to unfavorable genetic interactions. It is interesting to note in this context, that in the X-chromosomal haplo-diploid situation in *Drosophila* synthetic, lethal combinations do not occur[23].

1 We thank R. Grantham for his criticism, J. David and J.M. Legay for reading the manuscript. The laboratory is associated with CNRS No 243.

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Giemsa centromere staining of *Humulus lupulus* L. metaphase chromosomes

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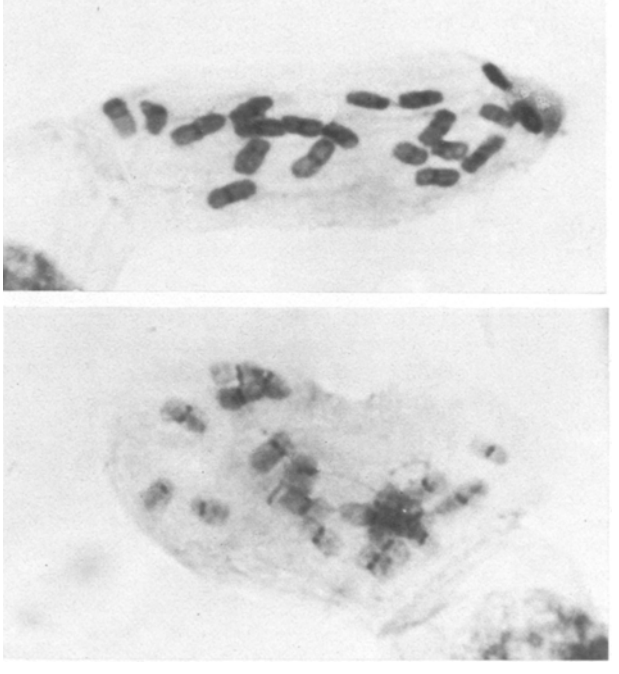
Summary. Giemsa staining of hop (*Humulus lupulus* L.) chromosomes at metaphase revealed kinetochore-like structures in the centromeric region.

Pardue and Gall[2] were the first to demonstrate the differential staining of mouse chromosomes with Giemsa stain. Since then, numerous workers have demonstrated the differential staining of animal chromosomes with Giemsa; the types of bands obtained have been designated as G-bands, C-bands, R-bands etc. These techniques have made it possible to identify different chromosomes and to detail their morphological features. The different Giemsa banding techniques were initially applied to animal chromosomes only, as plant chromosomes posed some difficulties. By now many workers have perfected these techniques on plant chromosomes also[3-5]. In plants C-banding has been demonstrated clearly, but there is no report of G-banding yet[6]. C-banding reveals the centromeric type of constitutive heterochromatin. In this paper an attempt was made to demonstrate the centromeric heterochromatin in *Humulus lupulus* L. metaphase chromosomes.

Materials and methods. Root tips were collected from the field during morning hours, thoroughly washed with distilled water and pre-treated in 0.05% aqueous colchicine for 4–5 h. The fixation was carried out in 1:3 acetic acid-alcohol for 24 h, followed by storage in 70% alcohol. The tips were hydrolyzed in 0.2 N HCl for 10 min at 60 °C and then washed thoroughly with distilled water. Squashing was performed in 45% glacial acetic acid. The cover slips were removed in 95% alcohol and air dried. The air-dried cover slips were kept for 2 days as such and then were placed in 0.007 N NaOH for 2 min at room temperature. After removal from NaOH, they were washed for about 10 min with distilled water and air dried. The air-dried cover slips were incubated in 2×SSC (0.3 M NaCl+0.03 M trisodiumcitrate, pH 7.0) at 60 °C for 45 min, then washed with distilled water for about 15 min, air dried and kept overnight. The staining was performed in Giemsa (E. Merck) diluted 50 times with M/15 Sörensen's phosphate buffer (pH 6.8) at room temperature. The optimum staining takes place in about 30 min. Then the cover slips were washed with distilled water, air dried and mounted in Euparal. All the solutions were prepared freshly.

Results and discussion. The diploid chromosome number of *H. lupulus* L. is 2 n = 20. In conventional staining techniques like Feulgen and aceto-orcein, all the 20 chromosomes show an unstained gap in the centromeric region whereas the Giemsa technique produced 2 darkly stained dots (figure). Giemsa Cd-banding technique of Eiberg[7] has revealed 2 dots in the region of the centromere in human chromosomes. Evans and Ross[8] have concluded that the Cd-bands are essentially the kinetochores. It has now been shown by electron microscopic studies that the centromere consists of 2 disc- or ball-shaped structures which are designated as kinetochores[9]. Stack[10], studying *Allium cepa, Ornithogalum virens, Tradescantia edwardsiana* and *Rhoeo discolor,* reports specific staining of the centromeric regions with Giemsa as paired dots and denotes them as kineto-



chores, as seen under the electron microscope. Marks[11] in *Nigella damascena* has also compared the paired centromeric Giemsa stained dots with the kinetochores. Our results also indicate kinetochorelike structures in *H. lupulus* L.

1 The authors are grateful to Dr C.K. Atal, for his keen interest and constant encouragement in the present study.

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Biochemical divergence between cavernicolous and marine Sphaeromidae and the Mediterranean salinity crisis

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Summary. Allozymic variation in proteins encoded by 14 loci was analyzed in 3 cavernicolous *Monolistra* and in 2 marine *Sphaeroma* species. Genetic distance data, high levels of heterozygosity and the divergence time calculations support the hypothesis that *Monolistra* diverged from its Sphaeroma-like marine ancestor during the Messinian, in connection with the Mediterranean salinity crisis.

The occurrence of Monolistrini (Crustacea, Isopoda), of the typically marine family Sphaeromidae in freshwater cave environments in continental Europe is a remarkable example of relict distribution. The present range of the cavernicolous species, all included in the genera *Caecosphaeroma* and *Monolistra,* extends from France to Herzegovina, including karst areas of the foothills of the Alps, Slovenia and Istria[2]. This distribution has been related to Miocene palaeogeography[3] in connection with the occurrence of deep, long-lasting inlets over these areas (figure 1). It has been speculated that the subterranean evolution of Monolistrini originated during the Miocene when their brackish water ancestors began to adapt gradually to freshwater environments[4]. This hypothesis and recent progress in the palaeogeography of the Mediterranean during the Tertiary suggested to us a study of the genetic structure and degree of divergence between 3 cavernicolous *Monolistra* and 2 marine *Sphaeroma* species. An attempt was made to identify *Sphaeroma* as a possible prototype for the marine ancestor of the cave-dwelling *Monolistra* by evaluating the divergence time between the 2 genera. The biochemical data presented here support this hypothesis.

The 2 *Sphaeroma* species selected for this study are both abundant and widespread in the Mediterranean[5]. *S. serratum* (Fabricius) is an intertidal species, whereas *S. hookeri* Leach lives in brackish waters[6] and may represent an adaptively intermediate stage in the evolution from marine to cave-dwelling forms. Population samples for electrophoresis of these species were collected in 2 localities along the Tyrrhenian coast. 3 *Monolistra* population samples were taken from underground streams in limestone caves situated along the foothills of the Alps in northern Italy, respectively in prealpine Lombardy near Bergamo, *M. boldorii bergomas* Arcangeli, in the Colli Berici near Vicenza, *M. berica* (Fabiani) and in prealpine Friuli near Tarcento (Udine), *M. caeca* Gerstaecker.

Samples of about 50 individuals per population were assayed using current electrophoretic techniques[7]. Genetic variation was analyzed in enzymes and other proteins encoded by 22 gene loci. A minimum of 18 loci were assayed for each species. 14 loci were common to all the species studied and the indices of genetic distance were calculated on this basis. Other details of collecting sites and electrophoretic methods and results are available.

Table 1 shows the genetic distance between the various species, calculated by the Nei method[8]. Figure 2 shows the phylogenetic relations among the Sphaeromidae studied as revealed by our data. It is immediately clear that the distances between *Sphaeroma* and *Monolistra* are higher than those recorded for the congeneric species, either *Sphaeroma* or *Monolistra.* Also, among the *Monolistra* the 2 easternmost species, *M. caeca* and *M. berica,* show the highest degree of similarity. These findings are consistent with the greater part of available data on comparisons between true species and between different genera[9–11]. Estimates of absolute divergence time between pairs of Sphaeromidae species were made using Nei's formula[11]. We used 2 alternative values for α (figure 2). 2 reservations must be made, however, concerning this methodology. a) Nei's formula is correct only under certain assumptions about protein structure and evolution[12], and b), the standard errors in the genetic distance estimates are fairly large

Fig. 1. Present distribution of the Monolistrini (black areas) superimposed on the Middle Miocene Palaeogeography of the Mediterranean (reconstruction after Hsü et al., 1977). White areas: land; dotted areas: seas.