chaeta number, and probably for chaeta number elsewhere on the fly. Chaeta number traits seem to be controlled by polygenic activity mainly restricted to specific parts of the genome[4,5]. Thus it is necessary to commence with strains having the appropriate genes. Traits such as viability and fecundity are probably influenced by an enormous number of genes so that polygenic activity controlling them may be expected to be ubiquitous and less localized, leading perhaps to less heterogeneity and hence less variability in the response to directional selection. There is evidence that the genetic architecture of such traits, which in nature are mainly subject to directional selection, differs from those such as chaeta number which are subject to stabilizing selection[6,7], so that artificial directional selection will be expected to be less effective for traits normally subject to directional selection than those subject to stabilizing selection[8].

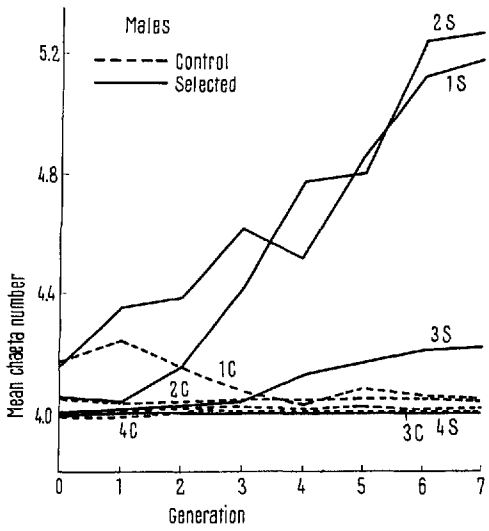

Fig. 2. The mean chaeta number in the 4 selection lines (S) and control lines (C) set up from populations as described in the text (males).

Résumé. Chacune de 16 souches, filiations de femelles uniques, et fécondées dans la nature, montrait une proportion caractéristique de mouches ayant plus de 4 soies scutellaires (soies supplémentaires). La sélection pour soies supplémentaires allait à une vitesse bien plus grande chez les lignées portant un haut niveau de soies supplémentaires que chez les lignées où ce niveau était plus bas. Ces résultats ont une répercussion importante sur la théorie des expériences de sélection.

SALLY M. W. HOSGOOD and P. A. PARSONS

School of Biological Sciences, La Trobe University, Bundoora, Victoria (Australia), 17 April 1967.

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Observations on the Centromere Area of Human Chromosomes

Uncertainty over the exact function and structure of the centromere area of chromosomes persists[1-5]. The area adjacent to the centromere often has a particularly bright, clear, slightly refractile appearance (Figure). GERMAN independently has made the same observation and commented that the chromatids in the area of exchange in somatic crossing over were at times distorted by a material similar in appearance to that which normally indents the centromere[6]. This appearance of the centromere area has not otherwise been commented upon or studied intensively. The term centromere area will be used to describe this area adjacent to the centromere and the term centromere will refer to the narrowed portion of the chromosome itself. It is the purpose of the present report to record certain observations on the behavior and appearance of the centromere area in cultured human lymphocytes.

Methods. The observations were made primarily by phase microscopy on air-dried human leucocytes stained with aceto-orcein after a culture period of 3 days as previously described[7]. Exceptions are included under observation. Acridine orange staining for RNA was carried out as described by GLUCK et al.[8] and DDD staining for SH groups as described by BARNETT et al.[9,10]. Monochromatic light was obtained with a green filter and a Zeiss mercury arc UV-light source.

Observations. A bright, clear, well-demarcated circle was present at nearly every centromere area in some cells (Figure a), and at few or none in other cells. Clear areas have been seen at the centromere of every chromosome, and although usually present bilaterally, at times may be either less evident or absent at 1 of the 2 centromere areas of a chromosome (Figure a). They were best seen by phase microscopy, but were also present on bright field examination as well as when observed with monochromatic light. When the bright appearance was present a

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concave appearance was usually imparted to the centromere, rather than a doubly convex pinching in. Sufficient staining of the cytoplasm with orcein or Wrights stain to provide contrast facilitated observation of this area, and the same clear bright appearance was present in orcein squashes as well as unstained air-dried cells. Rarely, the clear area was not in the shape of a circle but formed a linear streak extending out from the centromere for a distance twice the diameter of the chromatid. These streaks occasionally extended from a centromere to an adjacent secondary constriction or the area between the chromatids (Figure b). In the cells not treated with colcemide and cells in prophase this appearance was seen much less frequently, and the clear area was much smaller. Colchicinized cells not treated with hypotonic solution also showed the bright areas. Cells prepared without hypotonic treatment and colcemide were too poorly spread to permit observation of the centromere area. A very close association of these bright areas and even apparent fusion was often seen, both between acrocentric chromosomes in association (Figure c) and between the centromere areas of metacentric and submetacentric chromosomes (Figure d).

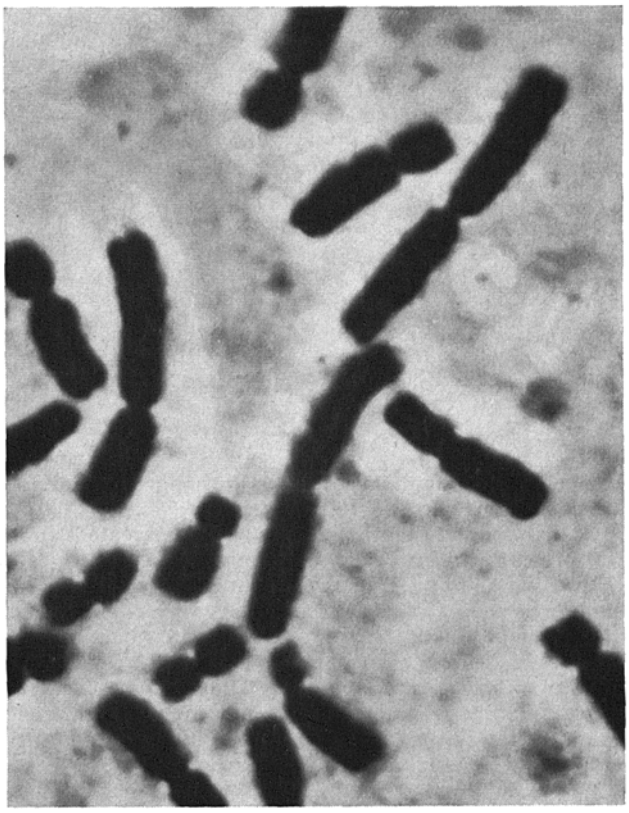
Occasionally, similar clearly demarcated but less refractile circles were present along the arms of the chromatids. They may either be adjacent to a straight arm or displace the arm and at times were seen adjacent to a secondary constriction.

Attempts to identify any material in the centromere area were unsuccessful. Particular efforts were made to identify RNA or spindle fiber material. Although the deep orange fluorescence characteristic of RNA when stained with acridine orange and examined with UV-light extended into the centromere area, the appearance was indistinguishable from the rest of the cytoplasm. Wright's stain delineated the area clearly but no positive staining was seen. No positive staining for sulfide groups with DDD was seen in the lymphocytes, although control rat skin fixed in methyl-acetic acid was positive. PAS staining was similarly negative in the lymphocytes, although positive in control rat liver cells.

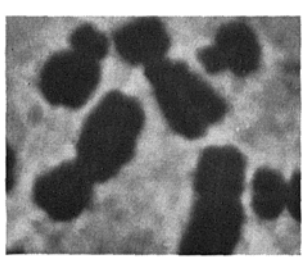
The appearance of the centromere could not be attributed to a specific fixative since the bright area was present using a variety of fixatives, but a non-specific effect of fixation could not be ruled out since even spreading directly from the media solution involved air drying. The bright areas were present in unstained metaphases observed by phase microscopy and therefore were not attributable to any of the staining procedures.

A similar appearance was present at the centromere of other species, where it was looked for. Bright areas were easily seen in cells from the golden hamster and rhesus monkey, but were smaller and more difficult to observe in chromosomes of the mouse. Many published photographs show the same appearance of the centromere.

Discussion. Several questions are raised by these observations, which cannot be answered with certainty. Of prime importance is the reason for the bright appearance adjacent to the centromere. It is possible that all or part of this appearance is due to diffraction of light by the identation at the centromere, but there are a number of points against this interpretation. In some chromosomes the clear areas were seen at only 1 of the 2 centromere areas which were otherwise apparently identical in shape (Figure a), and should, therefore, have produced similar diffraction of light. Where the arms of a short, symmetrical metaphase chromosome such as No. 19 have pulled apart to form a wide *X*, only 2 such areas were generally seen. The occasional linear streaking from the centromere constitutes the strongest argument that a collection of material or structure is present rather than an optical effect. There is, however, no proof that the



a



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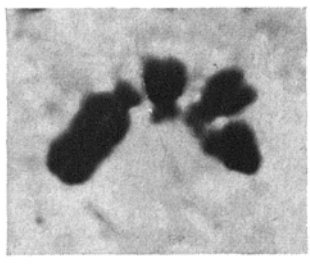

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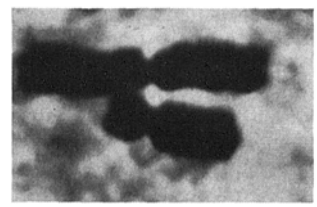

d

(a) The bright appearance of the paracentromere area is present at nearly every centromere. Only 1 of the 2 centromere areas of chromosome No. 1 (center), however, shows this appearance. Orcein stain. (b) Centromere streaks extending from the centromere of No. 9 to the terminal interchromatid area of adjacent No. 6 and to the centromere of a group *G* chromosome on the opposite side. (c) Association of centromere area bright spots in a group of acrocentric chromosomes. (d) Association of centromere areas of chromosomes No. 1 and group *C* chromosome.

streaks and light spots are chemically identical although their appearance is identical. The occasional presence of similar clear, bright areas along the arms of the chromatids is also not explained by diffraction. Lastly, the appearance of the centromere area was unchanged when observed by monochromatic light. Taken together, however, these points constitute sufficient evidence to make diffraction of light an unlikely cause.

Differential thinning or tearing of the cytoplasm during fixation, with a resulting increase in transmission of light in the centromere area is a second possible explanation. The occurrence of this appearance under a wide variety of conditions and fixation makes this unlikely. These conditions included aceto-orcein squashes, air drying following fixation with methyl-acetic acid and Carnoy's, as well as the sedimentation technique recently described by GAILLARD et al.[11] which permitted use of a fixative not containing alcohol or acetic acid (formaldehyde).

It is likely that both the bright spots adjacent to the centromere and the streaks extending out from the centromere, as well as the material noted by GERMAN to distort the chromatids in areas of somatic crossing over, are due either to spindle fiber material which was unstained by the procedures employed or to the structures recently observed in this area by electron microscopy[4,5]. The small size of the areas in question undoubtedly contribute to the difficulty in histochemical identification of the area by light microscopy, and in the absence of positive histochemical identification a final conclusion cannot be reached.

The appearance of the configuration shown in Figure c, as well as the association of the acrocentric chromosomes with the centromere of No. 1 in man[12] suggest that the centromere, as well as the nucleolus and secondary constrictions, plays a role in chromosome association[13].

Zusammenfassung. Das dem Centromer der menschlichen Chromosomen (ebenfalls bei andern Arten) anliegende Gebiet erscheint hell, klar und rund. Dies wurde in einigen Zellen bei fast jedem Centromer, bei andern Zellen nur selten oder überhaupt nicht beobachtet. Eine ähnliche Erscheinung trat auch in Form von Streifen auf, die sich manchmal vom Centromer aus zu anliegenden Chromosomen erstreckten oder sich ins Cytoplasma ausbreiteten. Die mögliche Deutung dieser Beobachtung wurde besprochen.

H. A. LUBS and SYLVIA L. BLITMAN

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Regulation of Tryptophan Metabolism in the Parasitic Wasp, *Habrobracon juglandis*

The synthetic pathway from tryptophan to the dark eye-pigments (ommochromes) has been analyzed by paper chromatography, fluorometric methods, and enzymatic assay. For quantitative determination of intermediate products, extracts of wild type o^+ (stock 33) and of the mutants *o* (orange eyes) and o^3 (for survey see [1]) were centrifuged and the supernatants chromatographed in *n*-propanol (70%) and Na-citrate (4%). Kynurenine, kynurenic acid, 3-hydroxykynurenine, and xanthurenic acid were identified as fluorescent spots (365 nm). The concentration of these substances during postembryonic development is shown in Figure 1 (for abbreviations see this Figure). There are higher concentrations of kynurenine in *o* than in o^+ at all stages of development. No 3-hydroxykynurenine is synthesized in *o*. Even the eggs of the mutant *o* contain more kynurenine than do those of wild type. This is due to predetermination. The mutant o^3 accumulates kynurenine at the same rate as *o*, except in ♂♂ of 3-day-old pupae ($X \pm t_{(\alpha=2\%;\, f=8)} \times s_{\bar{x}} = 8.9 \pm 1.89$ fluorometric units/2 p_3 of the mutant *o* and 13.1 ± 2.1 fluorometric units/2 p_3 of the mutant o^3). There is still no explanation for this significant difference.

In *Habrobracon* tryptophan is degraded via kynurenine to kynurenic acid and via 3-hydroxykynurenine to xanthurenic acid[2]. Whereas the orange-eyed mutant lacks xanturenic acid, both substances are found in o^+. It has been proved that kynurenine is rapidly metabolized to 3-hydroxykynurenine in fl_3 and sl. Therefore the concentration of kynurenic acid is lower at these stages than the concentration of xanthurenic acid (Figure 1). A high level of kynurenine in the mutant *o* or in wild type which had been fed tryptophan (by injection into the Ephestia hosts, mutant *a*) yields the large amount of 1 γ kynurenic acid/5 sl of *o* respectively, 2 γ/5 sl of wild type. Nearly all kynurenic acid and xanthurenic acid is excreted during the moult to prepupa.

In spite of high concentrations of kynurenine and 3-hydroxykynurenine at the pupal stages, there are very low but constant amounts of kynurenic acid and xanthurenic acid at this time of development. It seems, therefore, that only in fl_3 and sl there are strong in vivo activities of kynurenine and 3-hydroxykynurenine transaminase. It is still unknown whether these reactions are performed by 2 specific transaminases or by 1 transaminase which is less specific for kynurenine than for 3-hydroxykynurenine.

Enzymatic assays (Figure 2, II and III) of crude extracts show, however, low transaminase activities in old feeding larvae and spinning larvae and high activities in 2-day-old pupae and in adults. This result is in contrast to the assumed high in vivo activity at fl_3 and sl and the very low activity at pupal and imaginal stages. Therefore, it is thought that substrate concentrations are less responsible for the contrasting results in vivo and in vitro

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