

ISSN 0870-7235

BROTÉRIA GENÉTICA

REVISTA QUADRIMESTRAL

ÓRGÃO DA SOCIEDADE PORTUGUESA DE GENÉTICA

Subsidiada pela

**Junta Nacional de Investigação Científica
e Tecnológica**



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CONDIÇÕES DE ASSINATURA PARA 1993

Portugal: Esc.: 1.250\$00 (oferecida gratuitamente pela Sociedade Portuguesa de Genética aos seus sócios)
Outros Países: Dol. \$17.00
Número avulso: Esc. 500\$00

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Comp. e Imp. – Gabinete Comercial Gráfico, Lda.
Rua dos Duques de Bragança, 6 – 1200 LISBOA
Depósito Legal n.º 23964/93

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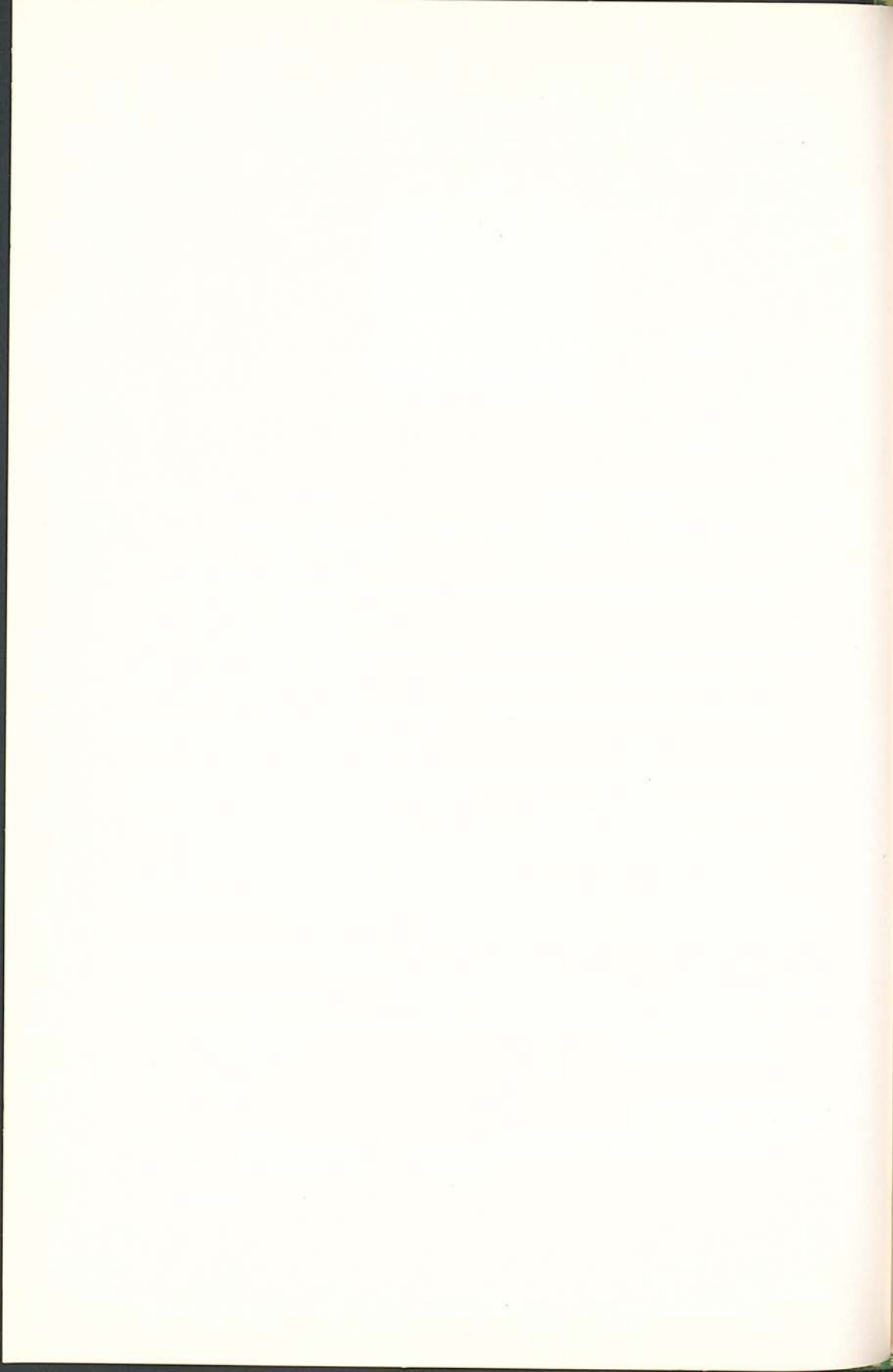
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A IMPORTÂNCIA DE SER BACTÉRIA

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ABSTRACT

A heterogeneous group of cellular organisms, bacteria have been successively placed together with Animals, Plants and Protists, only to recently be given a kingdom of its own (*Monera*, Procaryonts), and later on to be split into two superkingdoms or domains (*Eubacteria*, *Archaeobacteria* / *Bacteria*, *Archaea*). The variety of their metabolism turns the biogeochemical cycles on, even in extreme environments. Bacteria are, probably, the ancestors of the eucaryontic cells, and their organelles, and play an important role in keeping them healthy, or disrupting their balance.

INTRODUÇÃO

Os primeiros, dentre os organismos a que hoje chamamos bactérias, terão sido descritos por Leeuwenhoek (1677, 1683), e incluídos nos ANIMAIS. Para ele, o movimento era muito importante para dada forma ser considerada viva, tanto que: "...Além do descrito, a maior parte dessa matéria [massa interdentária] consistia dum grande número de linhas, bastante diferentes umas das outras em comprimento mas todas da mesma largura; umas eram direitas, outras eram dobradas, e dispostas desordenadamente. E como, anteriormente, eu tinha visto pequenos animais vivos com a mesma figura, fiz todos os esforços para ver se nestes também havia vida; mas não consegui ver em nenhum deles o mínimo movimento que se parecesse com o que quer que fosse vivo..." (ver Madeira-Lopes 1982).

Já Cohn (1875), com base em muito mais formas, achava que as bactérias deviam ser consideradas PLANTAS, pois "estão em relação directa e íntima com organismos [algas azuis, hoje cianobactérias] que são indiscutivelmente algas". A própria existência das formas mais pequenas seria duvidosa se elas não ocorressem em números tão grandes".

Entretanto, em 1866, tinha Haeckel passado as bactérias para os PROTISTA, reino que compreendia organismos que não eram nitidamente animais nem nitidamente plantas (algas, protozoários, espongiários, fungos e bactérias). Há aqui a disparidade da concorrência de duas classificações na mesma época, em que a mais avançada foi proposta antes e não reconhecida, pelo menos inicialmente. O mesmo acontece presentemente, e com muito menos razão, pois a comunicação não é problema, só a casmurrice é! Além disso a proposta de Cohn foi levada a efeito em 1853, e apenas reafirmada em 1875.

MONERA foi um reino inventado por Copeland, em 1938, e utilizado por Whittaker (1969) na sua célebre classificação ainda hoje infelizmente utilizada quase universalmente, com ou sem pequenas modificações (Whittaker and Margulis 1978; Margulis and Schwartz 1987).

É que, em finais dos anos 60, início dos 70, o reconhecimento da diferente arquitectura das células bacterianas tinha sugerido a formação do super-reino dos PROCARIONTES, por oposição ao dos Eucariontes (contendo este todos os organismos não-bacterianos). Murray (1974) descreveu então as características citológicas, ultraestruturais, bioquímicas e biofísicas usadas na distinção dos dois tipos de organização celular (Madeira-Lopes 1982, 1987, 1991b).

DEFINIÇÃO

Bactérias são um grupo heterogêneo de organismos (para generalidades, ver Madeira-Lopes 1987, 1991b):

a) celulares (geralmente unicelulares, alguns pluricelulares);

b) de volume geralmente inferior a $50\mu\text{m}^3$ (correspondente a esferas até $4\mu\text{m}$ de diâmetro (*Sarcina*), cilindros – como *Bacillus* – ou hélices – *Spirillum* – até $2\mu\text{m}$ de diâmetro e $10\mu\text{m}$ de comprimento); no entanto, bactérias muito maiores têm sido descritas: *Beggiatoa*, uma bactéria sulfuricante (usa H_2S como dador de electrões numa respiração aeróbia), surgiu em formas ovóides com $120\mu\text{m}$ de comprimento (Jannasch 1989 et al.); *Spirochaeta plicatis*, uma hélice com $0,75\mu\text{m}$ de diâmetro e até $250\mu\text{m}$ de comprimento, contendo várias células (Blakemore and Canale-Parola 1973); *Epulopiscium fishelsoni*, um habitante do intestino do peixe-cirurgião, atinge $600\mu\text{m} \times 80\mu\text{m}$ (Fishelson et al. 1985, Angert and Clements 1993); a **Fig. 1** mostra as dimensões relativas de quatro bactérias;

DIMENSÕES DAS BACTÉRIAS

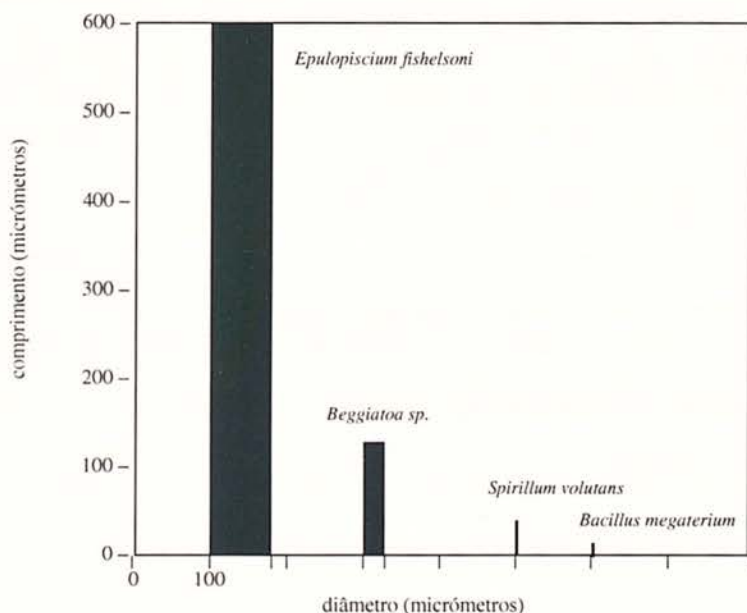


Fig. 1 – Comparação das dimensões de quatro bactérias. A maioria das bactérias conhecidas tem dimensões inferiores a *Bacillus megaterium*.

c) a maioria com um tipo característico de parede celular, contendo peptidoglicano (eubactérias), ou não (arqueobactérias), e algumas sem parede (Doyle 1992);

d) com flagelos, quando presentes, do tipo 1 (Macnab and Parkinson 1991), e não $2 \times 9 + 2$ como os flagelos e os cílios dos eucariontes (Blair and Dutcher 1992); por exemplo, os flagelos do gigante *Epulopiscium* são indicativos da sua condição bacteriana (Clements and Bullivant 1991); certas bactérias com forma de hélice flexível (espiroquetas) possuem flagelos interiores (filamentos axiais);

e) com diferenciação celular, típica de certos gêneros (ver Madeira-Lopes 1991b):

e1) desdiferenciável – como endósporos (cujo desenvolvimento tem sido objecto de interessantes trabalhos – Strauch and Hoch 1992, 1993), por vezes acompanhados de corpos paraspóricos (insecticidas biológicos muito específicos), conídios, cistos, acinetas, todos eles capazes de voltar à condição vegetativa;

e2) ou não-desdiferenciável – como bacteróides, heterocistos, ambos fixadores de azoto atmosférico – que não voltam à prévia condição vegetativa;

f) com reduzido grau de diferenciação do sistema membranoso (sem envólucro nuclear, mitocôndrias, cloroplastos, retículo endoplásmico ou aparelho de Golgi), mas em certos organismos com mesossomas, vesículas fotossintéticas, vesículas nitrogenásicas; a

bactéria *Epulopiscium* é rica em membranas (contínuas com a membrana celular) mas o seu núcleo é desprovido de envólucro (Clements and Bullivant 1991), o que não acontece, em contrapartida, no organismo *Gemmata obscuriglobus* que, apesar de possuir envólucro nuclear, é considerado bactéria pela sequência nucleotídica do seu rRNA 16S (Fuerst and Webb 1991);

g) com um único sistema de síntese proteica ribossômica, sendo os ribossomas do tipo 70S (com subunidades 30S e 50S), e rRNAs 5S, 16S e 23S; nesta alínea também caberiam os protozoários *Giardia* e *Vairimorpha* que foram colocados nos eucariontes por terem envólucro nuclear (apesar da ausência de mitocôndrias, peroxissomas ou aparelho de Golgi) e, mais importante, pelas sequências dos seus rRNAs (Madeira-Lopes 1988b, 1989);

h) com sequências nucleotídicas características nos rRNAs 16S e 23S, que as separam dos eucariontes (Olsen and Woese 1993);

i) com projecções características das subunidades dos ribossomas (Lake et al. 1984).

Só em bactérias se tem encontrado (para generalidades, ver MadeiraLopes 1985b, Gottschalk 1986):

j) diazotrofismo (utilização de azoto atmosférico como fonte de azoto), detectado em 80 géneros de eubactérias e em 3 de arquebactérias (Elkan 1992); com objectivos de fertilização de solos, estimula-se a fixação de N_2 em *Rhizobium* (Temprano 1992) e em cianobactérias (Rodríguez and Guerrero 1992);

k) respiração anaeróbia (em que o receptor final de electrões é diferente do oxigénio): nitrato, sulfato, enxofre, dióxido de carbono, ferro férrico, uranio U(VI) (Lovley et al. 1991), outros iões metálicos oxidados (Blaut et al. 1992, Stewart 1992);

l) respiração aeróbia com dador de electrões inorgânico (litoquimiotrofismo), como amoníaco, nitrato, sulfureto, enxofre, tiosulfato, sulfito, hidrogénio, monóxido de carbono, ferro ferroso, outros iões metálicos reduzidos (Paul and Clark 1989, Kelly 1990, Nicholls and Ferguson 1992);

m) fotossíntese dependente de pigmentos diferentes de clorofilas (bacterioclorofilas e bacteriorrodopsinas), em anaerobiose (Rao et al. 1985), e até (em bactérias aparentadas com metilotróficas e com diazotróficas) em aerobiose (Harashima et al. 1989);

n) autotrofismo em que a fixação de CO_2 se realiza por um processo diferente do ciclo de Calvin: ciclo de Krebs invertido, sequência das metanobactérias, sequência dos eó-citos (Fuchs and Stupperich 1985, Fuchs 1989);

o) autotrofismo desligado de fotossíntese (ligado a respirações, com dadores de electrões orgânicos ou inorgânicos) – ver Madeira-Lopes 1988a;

p) heterotrofismo desligado de organoquimiotrofismo (ligado a fototrofismo, ou a litoquimiotrofismo – respirações aeróbias ou respirações anaeróbias);

q) capacidade de vida em condições adversas (extremofilismo), em que as estruturas energéticas celulares podem exibir interessantes variações (Krulwich and Ivey 1990);

ql) contrariamente às leveduras, em que a temperatura máxima de crescimento encontrada (em *Hansenula polymorpha*) foi de 48°C (Madeira-Lopes 1992), o crescimento du-

ma estirpe da arqueobactéria *Methanopyrus* situou-se numa gama de temperaturas entre 85°C e 110°C (Huber et al. 1989), enquanto que a eubactéria *Thermotoga* (Pool 1990) foi até aos 90°C; não foram no entanto confirmadas notícias de organismos vivendo a temperaturas até 250°C combinadas com altas pressões (Madeira-Lopes 1984); são presentemente utilizadas já enzimas de várias bactérias termófilas extremas, como a DNA – polimerase de *Thermus aquaticus* (Saiki et al. 1988), e muitas outras (Cowan 1992);

q2) o psicofilismo está também presente em bactérias, que foram isoladas de regiões polares (a -20°C) e da estratosfera (a 2700m e -40°C);

q3) extremófilas são ainda as halobactérias, capazes de se multiplicarem em meios com concentrações saturantes de NaCl, cujo estudo augura potenciais aplicações biotecnológicas (Rodríguez-Valera 1992);

q4) as alcalifílicas, tolerando valores de pH até 11,5; as acidófilas, como *Acidianus infernus*, crescendo a pH=1 e temperaturas de 96°C;

q5) as barófilas, necessitando de pressões de, pelo menos, 1 atmosfera, com óptimo a 300 atmosferas, e podendo ir até 1400 (Pool 1990, DeLong 1992);

q6) as resistentes a radiações ionizantes e ultravioletas curtas, como *Deinococcus* e *Deinobacter* (Smith et al. 1992);

r) um papel ímpar nos ciclos biogeoquímicos, nomeadamente no ciclo do azoto e no ciclo do enxofre, que só reacções mediadas por bactérias conseguem fechar; contrariamente à crença geral, que baseia todo o ambiente nas relações entre animais e plantas, o fino tecido do equilíbrio ecológico fundamenta-se, essencialmente, no variado metabolismo mediado por bactérias:

r1) algumas bactérias nitrificantes (*Nitrosomonas europaea*, por exemplo), são também metanotróficas (capazes de utilizar metano como fonte de carbono e de energia), mas somente em condições de baixo teor do ião amónio (<15ppm), o que geralmente não acontece em solos fertilizados artificialmente (Stuedler et al. 1989); mais do que CO₂, CH₄ é um forte gás no efeito de estufa, com um tempo de residência de 8-10 anos, e tem-se encontrado aumento da sua concentração na troposfera;

r2) outro exemplo: a recente eutrofização do Guadiana (Abril de 1993) pela simbiose feto *Azolla*-cianobactéria *Anabaena* pode ter sido desencadeada por um aumento de sulfato, libertador de fosfato dos sedimentos (Caraco et al. 1989); o fosfato será o factor limitante do crescimento do conjunto, uma vez que o azoto está garantido pela fixação de N₂ nos heterocistos da cianobactéria (Paul and Clark 1989), e o carbono pela fixação de CO₂ nas células vegetativas dos dois simbiontes; também a variação da concentração de sulfato depende do equilíbrio entre a sua formação e o seu desaparecimento, ambos (não só mas também) mediados por bactérias (Madeira-Lopes 1985b, 1988a).

CLASSIFICAÇÃO

As bactérias têm sido classificadas com base em critérios morfológicos (desde Cohn 1875), necessidades energéticas e de fontes de elementos, utilização de certos compostos,

presença e posição de flagelos, coloração de Gram (que depende da constituição da parede), e estes critérios continuam a constituir o fundamento da última edição do Manual de Bergey (Murray et al. 1984, 1986, 1989). Só que, contrariamente à edição anterior (Buchanan et al. 1974), houve alguma preocupação em separar as arqueobactérias numa secção, dentre as 33, mas sem lhes dar o estatuto de super-reino, ou de domínio, como se vem impondo há pelo menos uma década! Outra inovação do novo Manual foi a de incluir as cianobactérias, o que tinha já sido prometido anteriormente. Dificuldades várias, que se prendem por vezes em conjugar conceitos antigos com ideias emergentes, levaram, por exemplo, a colocar as halobactérias na Secção 25 – Arqueobactérias, e também na Secção 4 – Cilindros e esferas aeróbios Gram negativos; já agora, por que não também na Secção 5, dos anaeróbios facultativos, ou nas Secções 18 e 19, dos fotossintéticos?

Não há ainda possibilidade duma classificação global de cariz molecular (Kandler 1985) que englobe a maioria das bactérias conhecidas, mas a classificação, muito fragmentada, proposta por Woese e colaboradores (Woese et al. 1990, Olsen and Woese 1993) constituirá provavelmente o núcleo donde essa futura classificação irá partir (Fig. 2).

EVOLUÇÃO

A árvore filogenética da Fig. 2, foi deduzida pela aplicação de metodologia estatística e lógica, como a análise de parsimónia (Caro-Beth 1993), a resultados experimentais de

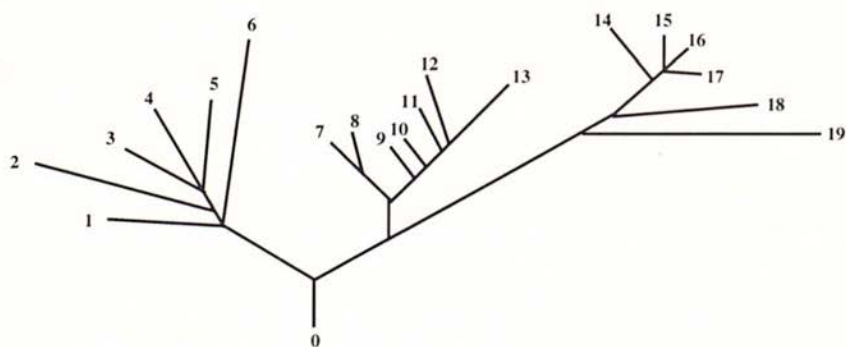


Fig. 2 – Árvore filogenética dos organismos celulares (Woese et al. 1990, Olsen and Woese 1993).

0 – Progenote
EUBACTÉRIAS (BACTERIA)

- 1 – Termotogales
- 2 – Flavobactérias
- 3 – Cianobactérias
- 4 – Bactérias roxas
- 5 – Bactérias Gram positivas
- 6 – Assullobactérias verdes

ARQUEOBACTÉRIAS (ARCHAEA)

- 7 – *Pyrodictium*
- 8 – *Thermoproteus*
- 9 – Termococales

- 10 – Metanococales
- 11 – Metanobactérias
- 12 – Metanomicrobiales
- 13 – Halobactérias

EUCARIONTES (EUCARYA)

- 14 – Animais
- 15 – Ciliados
- 16 – Plantas
- 17 – Fungos
- 18 – Flagelados
- 19 – Microsporídeos

sequenciação monomérica de polímeros (nucleótidos dos rRNAs, essencialmente), enquanto que a sua raiz foi fixada por uma estratégia, proposta por Schwartz e Dayhoff (1978), da análise da hipotética duplicação génica (inferida de sequências parálogas) anterior à formação de cada ramo primário (Olsen and Woese 1993). Aí se situa o antepassado universal, o progenonte (Woese 1987, Sogin 1991), entidade teórica com potencial para evoluir em células já semelhantes às de hoje.

O padrão fenotípico do ramo eubacteriano aponta para o carácter primitivo da fotossíntese (Madeira-Lopes 1986c) e do termofilismo, este também apontado para as primeiras arqueobactérias (Noll 1992) juntamente com a anaerobiose e o litoquimiotrofismo (respiração com dador inorgânico). Ambos os ramos seriam originalmente autotróficos e não heterotróficos, como foi inicialmente sugerido por Oparin e adoptado universalmente (Woese 1979, Loomis 1992).

Presentemente com reduzido grau de aceitação, a proposta evolutiva de Lake e colaboradores (Lake et al. 1984, 1985, Madeira-Lopes 1986b), inicialmente baseada nas projecções (observadas em microscopia electrónica) das subunidades dos ribossomas mas também estendida (com diferente metodologia interpretativa) aos rRNAs, indica uma origem comum para eubactérias e halobactérias, grupo apelidado de fotócitos (ver Madeira-Lopes 1987), e outra para metanobactérias, eucariontes e eócitos – nome este cunhado por Lake para as arqueobactérias dependentes de enxofre elementar, os Crenarchaeota de Woese (Woese et al. 1990). De qualquer modo, também para Lake, a fotossíntese seria carácter primitivo (Brock 1989).

Ainda não suficientemente exploradas, mas de aparente potencial filogenético, são as sequências aminoacídicas das várias polimerases: DNA, RNA, transcritase inversa (Palenik 1992).

Também as H⁺-ATPases têm fornecido algumas achas para a fogueira: as do tipo F estão presentes nas membranas celulares de eubactérias e nas membranas interiores das organelas eucarióticas (mitocôndrias e cloroplastos), enquanto que as do tipo V se encontram no sistema endomembranoso de eucariontes (vacúolos, lisossomas, endossomas, aparelho de Golgi, etc.) e em arqueobactérias. Um esquema evolutivo propõe que uma ATPase-V progenônica (Fig. 2, 0=Progenonte) evoluiu para três ATPases, duas V (uma arqueobacteriana e uma proto-eucariótica, que deu a vacuolar), e uma P (eubacteriana) que, por sua vez, originou as mitocondriais e as cloroplásticas (Taiz and Nelson 1989, Marquis 1992).

ASSOCIAÇÃO

As bactérias associam-se umas com as outras e também com eucariontes. Em equilíbrio com as cerca de 10¹³ células eucarióticas dum animal, como o homem, haverá umas 10¹⁴ células bacterianas (Tannock 1988), que normalmente impedem a proliferação de patogénicos (Kaufmann and Embden 1993, Tilney and Tilney 1993). Bactérias associadas com a partenogénese em insectos, evitam a segregação cromossómica em óvulos ou interferem com a cariogamia em zigotos (Stouthamer et al. 1993).

Têm-se encontrado indícios de transferências génicas de eubactérias entre si, de eubactérias para arqueobactérias e para vários eucariontes, como fungos, animais e plantas (Heinemann 1991, Sprague 1991), e de eucariontes para eubactérias (Smith et al. 1992).

Esta transferência génica horizontal, também se tem detectado entre as organelas eucarióticas: DNA das mitocôndrias para o núcleo e dos cloroplastos para as mitocôndrias (Madeira-Lopes 1983), possivelmente mediada por RNA (Brennicke 1992).

Os transposões são pedaços de DNA viajantes no mesmo ou entre cromossomas (Madeira-Lopes 1986a), podendo fazer essas viagens por intermédio de RNA (retrotransposões): existe apoio experimental da transferência de transposões entre células eucarióticas (Kidwell 1992).

Os intrões (sequências intragénicas, posteriormente excisadas), inicialmente descobertos em eucariontes (Archer 1980, Woolford and Peebles 1992, Stoltz and Doolittle 1993), e posteriormente em arqueobactérias (Madeira-Lopes 1987), foram também já descritos em eubactérias (Doolittle 1991). Uma proposta de filogenia dos intrões (Cavalier-Smith 1991) envolve a sua transferência de eubactérias primitivas para arqueobactérias, para arqueozóários (eucariontes primitivos, ramo 19 da Fig. 2) e para metacariontes (os restantes eucariontes), enquanto que, para outros (Palmer and Logsdon 1991), os intrões (pelo menos os nucleares) teriam tido uma origem mais recente.

A origem endossimbiótica dos eucariontes tem extenso apoio experimental (Gray and Doolittle 1982, Sogin 1991, Zillig 1991):

A parte nuclear dos eucariontes terá provindo dum organismo aparentado com arqueobactérias primitivas (Madeira-Lopes 1984b, 1985a, 1987, 1988b, 1989), a que se terá associado uma eubactéria helicoidal flexível (com flagelos interiores): assim teria surgido um eucarionte do grupo 19 (Fig. 1), ainda sem mitocôndrias; estas ter-se-iam originado de eubactérias do grupo 4, às quais se juntaram outros endossimbiontes, aparentados com cianobactérias do grupo 3, dando os cloroplastos (Margulis 1981, 1992, Rennie 1992). Os suportes destes conceitos estão já muito bem cimentados em variados resultados experimentais: por exemplo, nas semelhanças dos rRNAs de cianobactérias, de proclorófitos e de cloroplastos (Bryant 1992). Há, no entanto, observações que apoiam a hipótese de que os cloroplastos de certos eucariontes vieram doutros eucariontes (com núcleo envolucrado) e cloroplastos já formados (Madeira-Lopes 1991a).

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ASSESSMENT OF SPECIFIC GENOTYPE x ENVIRONMENT INTERACTIONS IN *LUPINUS LUTEUS* L.

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ABSTRACT

In a network of *Lupinus luteus* L. yield trials, within the same "Equipotential Zone for cultivar yield pattern evaluation", the data analysis through the Joint Regression method was able to reveal significant differences of the relative cultivar yield patterns, although no significance were detected on most of the individual trials using the ANOVA conventional method. The Friedman test of the residues to the regressions, for a 95% confidence limit, as proposed in the Trimmed Regression Method, revealed, in some trials, an organized distribution of the residues for one genotype. This situation was identified as the result of a "specific interaction", expressed through the seed shedding of that cultivar. Therefore, with the present results, this method proved to be valuable for the identification of specific interactions in *Lupinus luteus* L.

RESUMO

Numa rede de ensaios de produção de tremocilha, incluída numa mesma "zona equipotencial de adaptação para a avaliação das produtividades relativas", a análise dos dados pelo método de "regressão conjunta" permitiu detectar diferenças significativas dos "padrões relativos de produtividade", embora não se tenham detectado diferenças significativas na maioria dos ensaios, pela avaliação pelo método convencional de ANOVA. A análise dos resíduos para as regressões, para um limite de con-

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fiança de 95%, tal como proposta no método das Regressões Conjuntas Rectificadas, revelou que, em três ensaios, os resíduos para a respectiva regressão, da cultivar 'Cardiga', eram excluídos da "família" de resíduos observados nesses ensaios. Esta situação foi posteriormente identificada como resultante de uma "interacção específica", expressa pela elevada taxa de deiscência desta cultivar, particularmente manifesta em condições de colheita tardia. O método revelou-se, deste modo, eficiente para a identificação de situações de interacção específica em *Lupinus luteus* L.

INTRODUCTION

In a previous paper (Gusmão *et al.*, 1992), it was shown how the "specific instability" (Eberhart and Russell, 1966) can be further analysed in order to assess the specific "genotype x environment" interaction situations.

Based on the joint regression method, first proposed by Mooers (1921) and latterly developed after Finlay and Wilkinson (1963), it was shown, for triticale relative cultivar evaluation (Gusmão *et al.*, 1992; Mexia *et al.*, 1992), how the Friedman test (Friedman, 1937) of the residues to the regressions can be used to assess the situations of specific genotype x environment interactions. In the present paper, we evaluate the effectiveness of this procedure to assess specific genotype x environment interaction in *Lupinus luteus* L. cultivars.

MATERIALS AND METHODS

In the present analysis we use the results, in five yield trials, of a cohort of five *L. luteus* genotypes: cultivars 'Refusa' and 'Cardiga' and advanced lines RM 202-P, RM 202-B and RM 102-B.

Field trials were designed in five randomized complete blocks and carried out in Portugal, within the same "equipotential zone for relative yield pattern evaluation" (Gusmão *et al.*, 1989); Pegões (1988/89, 1989/90 and 1990/91), Mirandela (1989/90) and Elvas (1989/90).

To test for specific interactions we used the method described in Gusmão *et al.* (1992).

RESULTS AND DISCUSSION

In Table 1, we present the Friedman test for the residues, to the joint regression lines, of each cultivar, within each trial, before and after exclusion of the cultivar 'Cardiga'. As it can be seen, the presence of specific interactions can be detected in Pegões (1988/89 and 1989/90) and Elvas (1989/90); the hypothesis of equal parental distribution of the residues of the different cultivars, in these trials, can be discredited at a 95% significance level.

Only the cultivar 'Cardiga' exhibited specific interaction in these trials. As it can be seen in Table 1, after exclusion of this cultivar, the weighted value of the χ^2 was reduced to a non significant level.

The situations of specific interaction were easily related to the sensitivity of the cultivar 'Cardiga' to the climatic determinants of the dehiscence of the pods. In fact, in the trials where specific interaction was statistically evident, a strong proportion of seed shedding was registered, exclusively for this cultivar.

TABLE 1

Friedman test for the residues to the joint regressions, within each trial, before and after exclusion of the cultivar 'Cardiga', and for the regressions assessed without this cultivar.

Place	Year	With the cv. 'Cardiga'						Without cv. 'Cardiga' χ^2
		Before exclusion			After exclusion			
		d.f.	χ^2	*	d.f.	χ^2	n.s.	
Pegões	1988/89	4	10.40	*	3	0.60	n.s.	0.36
Pegões	1989/90	4	10.24	*	3	3.00	n.s.	2.28
Elvas	1989/90	4	9.76	*	3	2.04	n.s.	2.04
Mirandela	1989/90	4	0.48	n.s.	-	-	-	0.60
Pegões	1990/91	4	8.80	n.s.	-	-	-	0.60

d.f. - Degrees of freedom

* - 95% significance level for the rejection of the hypothesis of equal parental distribution of the residues.

n.s. - Not significant.

The limited number of trials did not allow to proceed with the Trimmed Regression method; however, there was an increase of the determination coefficient (r^2), for the joint regressions assessed without the cultivar 'Cardiga' (Table 2) and, at the same time, it was verified a drastic reduction of the χ^2 value, in the Friedman test for the residues within each trial (Table 1), which confirm the relevance of this technique, once safeguarded the assumptions for its correct application in relative yield trial evaluation (Gusmão, 1985; Gusmão *et al.*, 1989), notwithstanding the fact of having only five trials.

TABLE 2

Joint Regressions ($y = a + b x$), including and excluding the cultivar 'Cardiga'.

Genotype	Including the cv. 'Cardiga'			Excluding the cv. 'Cardiga'		
	α	β	r^2	α	β	r^2
RM 202-P	119.571	1.072	0.941	24.202	1.068	0.956
'Refusa'	-16.677	1.063	0.926	-111.707	1.060	0.942
'Cardiga'	-361.415	0.992	0.765			
RM 202-B	176.306	0.835	0.871	108.600	0.827	0.873
RM 102-B	82.206	1.038	0.873	-21.360	1.045	0.904

r^2 - Determination coefficient

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ELECTROFORESE EM GEL DE POLIACRILAMIDA-SDS DAS PROTEÍNAS DE RESERVA DE TRIGO (aperfeiçoamento de um método)

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SUMÁRIO

No presente trabalho foi experimentado o método de electroforese unidimensional descrito por Galili e Feldman (1983) para a separação das proteínas de reserva de trigo. Algumas modificações foram introduzidas nesta técnica com o objectivo de melhorar a resolução dos electroforegramas. As alterações experimentadas foram as seguintes:

- 1) – Uniformização das amostras por determinação do teor de humidade relativa do grão;
- 2) – Determinação da espessura óptima das placas de gel;
- 3) – Realização da electroforese a temperatura e intensidade de corrente constantes;
- 4) – Modificação nos processos de coloração e descoloração dos geis.

Conclui-se que as modificações introduzidas no método contribuiriam para uma melhor resolução do electroforegrama.

SUMMARY

The method of one dimensional electrophoresis for storage protein in wheat as described by Galili and Feldman (1983) was studied. Several modifications were made to the technique in order to improve its resolution capacity, as follows:

- 1) – Induced uniformity of the grain's relative humidity before electrophoregram determination;

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- 2) – Determination of the optimal thickness of the gel plates;
- 3) – Carrying out electrophoresis at constant electrical current and temperature;
- 4) – Modification in the staining and destaining methods.

It was concluded that the changes we made contributed for the improvement of the method.

INTRODUÇÃO

A concentração e a composição das proteínas de reserva no grão de trigo, estão dependentes da cultivar e das condições de cultura.

A constituição proteica do grão de trigo é um factor determinante na utilização de uma farinha como matéria prima para panificação ou para produção de massas alimentícias (Bietz et al., 1973; Law e Payne, 1983).

As proteínas de reserva do grão de trigo, designadas genericamente por prolaminas dividem-se em Gliadinas e Gluteninas; as Gliadinas conferem extensibilidade à farinha enquanto as Gluteninas determinam a sua elasticidade (Blackman e Payne, 1987).

O desenvolvimento de técnicas precisas de análise química e de manipulação cromossómica, permitiu obter informação detalhada acerca dos genes responsáveis pela síntese destas proteínas, o que tem contribuído para a determinação da qualidade da farinha.

A bioquímica e a genética das proteínas do glúten, têm sido extensivamente estudadas nestes últimos anos. Um considerável progresso foi obtido neste campo, pela aplicação da técnica PAGE-SDS (electroforese em gel de poliacrilamida-dodecil sulfato de sódio). Esta técnica contribuiu para um estudo mais detalhado do número, distribuição e controlo genético de prolaminas específicas.

Na maioria das técnicas unidimensionais PAGE-SDS, apenas uma das fracções de prolaminas (gluteninas ou gliadinas) do albúmen de trigo é predominantemente separada. Neste trabalho, utilizamos um método que resultou da modificação da técnica sugerida por Galili e Feldman (1983), o que permitiu a obtenção de electroforegramas com uma separação equilibrada de sub-unidades de ambas as fracções, fornecendo uma informação mais completa do teor proteico de cada uma das cultivares analisadas.

As modificações introduzidas, abaixo descritas conduziram à obtenção de electroforegramas com boa resolução.

MATERIAL E MÉTODOS

MATERIAL

Os materiais utilizados foram linhas de trigo tetraploide, envolvendo ou não a substituição cromossómica (1D/1A), provenientes do cruzamento entre *Triticum turgidum* L. ssp. *durum* cv. 'Ld 222' e *Triticum aestivum* L. cv. 'Chinese Spring', obtidas no Laboratório de Citogenética do Instituto Gulbenkian de Ciência; *Triticum turgidum* L. ssp. *durum* cv. 'Ld 222'; *Triticum aestivum* L. cv. 'Chinese Spring' e *Triticum turgidum* L. ssp. *durum* 'Câmara'.

A linha 'Câmara' é um trigo tetraplóide que envolve uma substituição cromossômica (1D/1B) estabilizada, proveniente do cruzamento das cultivares 'Ld 222' e 'Chinese Spring', foi obtida por Mello-Sampayo (1973).

MÉTODOS

A técnica utilizada para a extração das proteínas do albúmen e subsequente fracionamento foi a sugerida por Galili e Feldman (1983) com as modificações necessárias para a realização da técnica e para a obtenção de electroforegramas com bom poder de resolução e perfeitamente reprodutíveis.

Relativamente ao processo de extração, procedeu-se à introdução de uma fase inicial no tratamento do material para estudo, que visou uniformizar todas as amostras, procedendo-se, para tal, à secagem em estufa (a temperatura de $80 \pm 2^\circ\text{C}$) dos grãos após a extração dos embriões

No que diz respeito ao método electroforético foram testados os seguintes factores:

- 1) Temperatura ideal para completo enchimento da placa e obtenção de uma polimerização uniforme e gradual (0; 5; 10; 15 e 20°C);
- 2) Tempo necessário a uma polimerização completa do gel (4; 8; 12; 16 e 18 h);
- 3) Espessura da placa de gel (0,75; 1,0; 1,5; 3,0 mm);
- 4) Condições ideais para o fraccionamento das proteínas no gel:
 - 4.1. – A diferença de potencial constante (100 e 150V) sem controlo de temperatura;
 - 4.2. – A diferença de potencial constante (100 e 150V), mantendo a temperatura constante (0; 5; 10; 15; 17; 20°C);
 - 4.3. – A intensidade da corrente constante (6,0; 10,0; 12,5 mA) sem controlo de temperatura;
 - 4.4. – A intensidade de corrente constante (6,0; 10,0; 12,5 mA), mantendo a temperatura constante (0; 5; 10; 15; 17 e 20°C).

No processo de coloração e descoloração, as placas foram directamente colocadas em solução corante (Coomassie Blue R 250 a 0,02% em TCA a 12,5%) e transferidas para nova solução com uma concentração superior de corante (0,05% de Coomassie), sendo em seguida descorada em TCA a 12,5%, depois em água destilada e desionizada e finalmente em solução de Blakesley (Payne 1981).

Quer na coloração, quer na descoloração, qualquer dos passos efectuados tiveram a duração de 24h, sendo os geis sujeitos a agitação permanente.

RESULTADOS E DISCUSSÃO

Os resultados obtidos estão apresentados na tabela I e II.

TABELA I

		TEMPERAMENTO (°C)																		
		0				5				10				15				20		
TEMPO (h)	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	18	+	++	+	-	+	+++	+	+	-	-	-	-	-	-	-	-	-	-	
		0,75	1,0	1,5	3,0	0,75	1,0	1,5	3,0	0,75	1,0	1,5	3,0	0,75	1,0	1,5	3,0	0,75	1,0	1,5
		ESPESURA (mm)																		

Correlação entre as três variáveis estudadas:

Temperatura (°C); Tempo de polimerização (h) e Espessura da Placa (mm), para otimização do processo de polimerização.

TABELA II

		TEMPERAMENTO (°C)						
		Sem controle	0	5	10	15	17	± 20
Diferença de potencial (V)	100	-	-	-	-	+	+	-
	150	-	-	-	-	+	++	-
Intensidade da corrente (mA)	6,0	-	-	-	-	-	-	-
	10,0	-	-	-	-	+	++	++
	12,5	-	-	-	+	+	+++	++

Correlação entre a Temperatura de migração electroforética (°C) e a Diferença de Potencial (V); e, entre a Temperatura de migração electroforética (°C) e a Intensidade da Corrente (A), na otimização do poder de resolução dos electroforogramas.

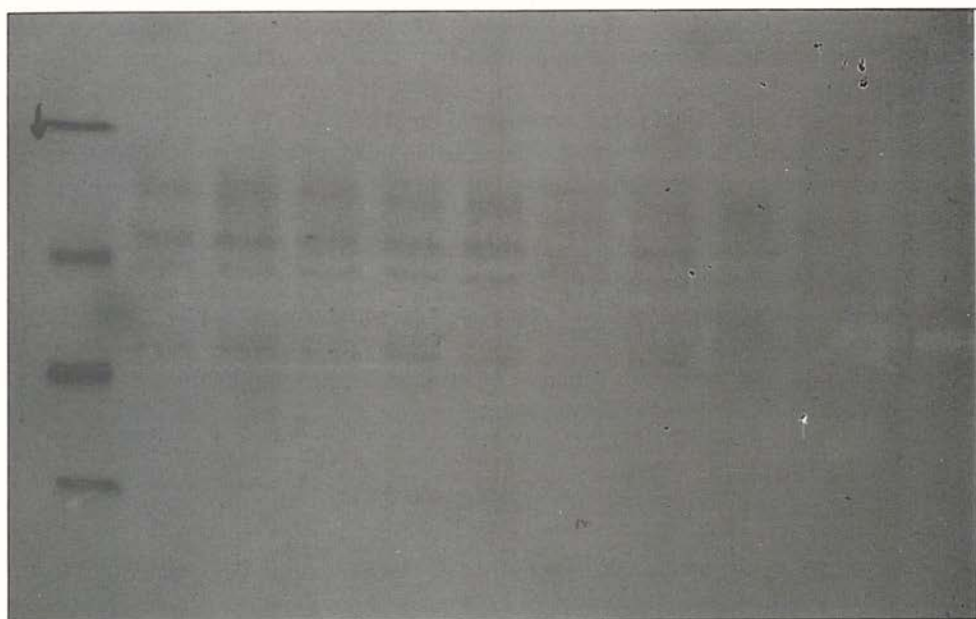


Fig. 1 – Resolução dos electroforegramas obtidos pela aplicação directa do método de Galili e Feldman (1983).

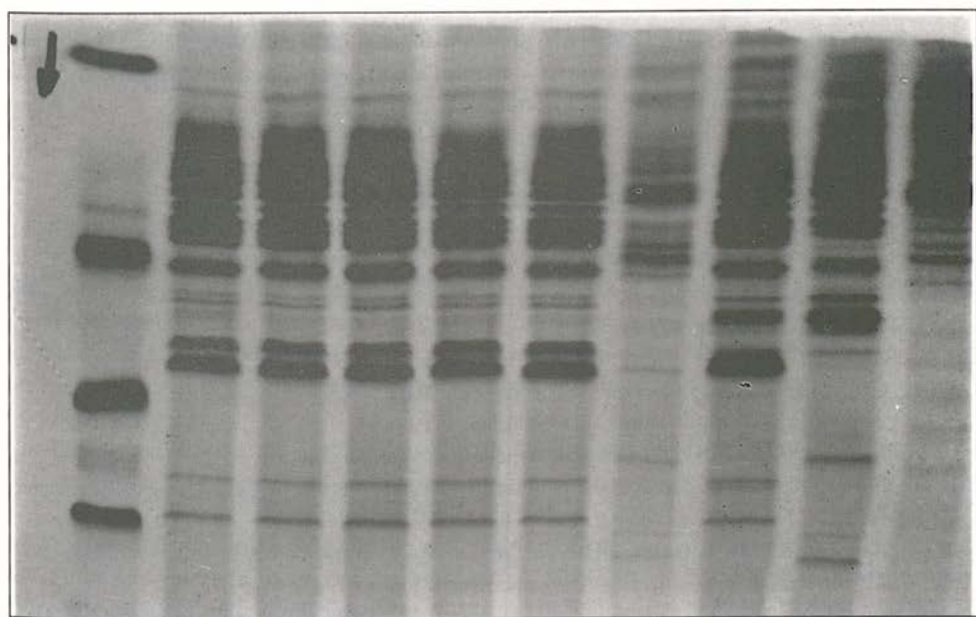


Fig. 2 – Resolução dos electroforegramas obtidos pela técnica modificada.

A secagem dos grãos teve como finalidade a uniformização do estado de hidratação das amostras, pois este depende das condições ambientais de cultivo e do processo de armazenamento.

A temperatura escolhida para o enchimento da placa foi de 0–5°C (tabela I). A temperaturas superiores a polimerização era demasiado rápida, dificultando o enchimento das placas e originando polimerizações pouco uniformes devido a correntes de convecção resultantes do excesso de energia térmica libertada no processo.

O tempo de polimerização deverá ser superior a 18h (tabela I), o que permite a obtenção de placas com bandas bem definidas e reprodutíveis.

A migração deverá ocorrer a uma intensidade de corrente constante: 12,5 mA, à temperatura de 17°C (tabela II).

Nestas condições a energia libertada é relativamente baixa, diminuindo, consideravelmente os efeitos prejudiciais que daí resultam.

CONCLUSÕES

Das alterações efectuadas, resultou que da técnica de Galili e Feldman (1983) unicamente se manteve o método de extracção e a composição dos geis.

Do aperfeiçoamento do método resultou a obtenção de bandas bem contrastadas num gel de fundo límpido, conforme se verifica pela observação da Fig. 2 comparativamente á Fig. 1 (método não modificado).

No electroforegrama da Fig. 2 estão bem definidas as bandas de gluteninas e gliadinas conforme o objectivo da técnica utilizada.

Vê-se com efeito que as gluteninas de elevado peso molecular se encontram expressas, bem como a gama extensa de gliadinas que as acompanham, todas com uma elevada definição.

Estudos posteriores ainda em curso, mostram a correspondência destas bandas relativamente ás que se obtêm pelos métodos correntes.

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Este trabalho foi efectuado no Laboratório de Citogenética do Instituto Gulbenkian de Ciência.

NEW BASIC CHROMOSOME NUMBER AND MEIOTIC ANALYSIS OF TWO SPECIES OF GENUS *HEDYOTIS* (RUBIACEAE).

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SUMMARY

Meiotic analysis of two species of *Hedyotis* (Rubiaceae), *H. paradoxa* shows $n=15$ and *H. alata* $n=13$. Both the species have been studied cytologically for the first time and the chromosome number reported here is the first report. Meiotic anomalies like cytomixis, laggard, multivalents and univalents have been observed and discussed.

INTRODUCTION

Hedyotis L. is a perennial wild shrub of Rubiaceae family. This is a quite large genus consisting of 150 species (Willis 1982) out of which 16 species are reported from Andaman and Nicobar Islands. Among the 16 species reported from Andaman and Nicobar Islands, one species *Hedyotis paradoxa* Kurz is endemic to Andaman and Nicobar Islands. Two more species have been recorded endemic to those islands but the plants have not been collected so far. *H. paradoxa* was abundant (Parkinson 1923) but in recent years it has been observed that the plant has become rare and threatened (Rao 1986). Cytologically the genus is very poorly known, only 23 percent species of the genus being known cytologically whereas among the species found in Andaman and Nicobar islands only two species are known cytologically that too from mainland India. Therefore, the cytology of the two species in question has been done for the first time with a view to know the chromosome number and their meiotic behaviour. Cytology of the genus is very interesting as the genetic chromosome number is varying from 11 to 36. In *Hedyotis articularis* R Br.(S) (Syn. *Oldenlandia articularis* Gamble) $n=11$ and in *Hedyotis nitida* W & A (Syn. *Oldenlandia nitida* (W & A) Gamble) $n=36$. Whereas in the present study chromosome number $n=13$ and $n=15$ is recorded.

MATERIALS AND METHODS

Flower buds of both the plants were collected at random from natural population (localities and population is mentioned in Table I) and were kept in saturated aqueous solution of para-di-chlorobenzene for 24 hours. Flower buds were then washed thoroughly in fresh water and, after removing all the water, buds were fixed in Carnoy's solution. A few drops of ferric chloride were added in the fixative as mordant to obtain good staining of the chromosomes.

Chromosome studies were made by squashing young anthers in 2 percent aceto-carmin and immediately microphotographs were taken from temporary slides. Pollengrains were analysed by staining mature anthers in 2 percent aceto-carmin. Stained pollengrains were scored fertile and unstained as sterile.

Voucher specimens of both the plants undertaken for present studies have been deposited in the herbarium of the Department of Botany, J.N.R. Mahavidyalaya, Port Blair (Andamans).

OBSERVATION

Hedyotis paradoxa Kurz and *Hedyotis alata* Koen are distributed in Andaman and Nicobar group of islands, but the former is endemic to these islands. Both are perennial wild shrub. Both are quite distinct morphologically. *Hedyotis paradoxa* was common in South Andaman but now it has become rare and threatened and localized in some small patches in deciduous forest, mostly under the shade or large trees.

Plant is large shrub, leaves sessile, oblanceolate, apex acuminate, inflorescence axillary cyme enclosing the nodes, flower white, dense flowered, fruit small, crustaceous, smooth, globose. It flowers from May to August.

TABLE I

Geographical locality, period of collection, chromosome numbers and percentage of sterile pollen.

Species with population	Period of collection & collection number.	Gametic chromosome number.	% of sterile pollen grains.
1) <i>Hedyotis paradoxa</i> Kurz. Hp 0590 South Andamans (Nayasahar)	May'90 (0099)	15	24.2
2) <i>Hedyotis alata</i> Koen Ha 1089 (Car Nicobar)	October'89 (0039)	13	18.6

Hedyotis alata has also reduced in number but it has not reached the status of rare and threatened plants, mostly the plant was found localized in the road side forest of the Car Nicobar.

The plant is small shrub, leaves are sub-sessile, obtuse, flower is white, few flowered, fruit small, four angled and four winged. It flowers round the year.

Chromosome counts of both the species are listed in Table I. This is the first report of chromosome number of both the species. Meiotic division in general was found to be synchronized and normal.

In *Hedyotis paradoxa* about 86 percent pollen mother cells were seen with normal 15 bivalents (Fig. 1) only in 10 percent PMC one or two quadrivalents and univalents were seen (Fig. 2-5). The phenomenon of translocation was seen clearly in 4.9 percent PMC. At anaphase I equal separation of chromosomes (15:15) were seen in most of the PMC. Only in 6.3 percent PMC laggards and in 9.6 percent PMC unequal separation of chromosomes were noticed. A very interesting phenomenon of cytomixis was observed at various phases of division but it was more acute at prophase stage and it was observed in 38 percent PMC at that stage. Consequently one additional patch of few chromosomes were seen at diakinesis and at metaphase I (Fig. 7). This patch was dividing separately and not mixing or pairing with the main set of chromosomes. In some PMC at metaphase I additional number of chromosomes were also seen. Some abnormal spore tetrad like monads, diads and triads were also noticed. Pollen stainability is reduced to 75.8 percent.

In *Hedyotis alata* more than 90 percent PMC at metaphase I were seen with normal 13 bivalents (Fig. 6 and 8) only few PMC were seen with multivalent and univalent. 9.3 percent pollen mother cells were seen with clumped chromosomes with precocious separation. Majority of the PMC at anaphase I were seen with equal (13:13) separation of chromosomes (Fig. 9). Laggards were seen in 8.7 percent PMC at anaphase I. Phenomenon of cytomixis was also seen in this species but the frequency was very less. It was in 11.2 percent PMC at prophase stage only. Abnormal spore tetrads were seen rarely. 81.4 percent pollen grains were found stained.

DISCUSSION

Meiotic analysis of *Hedyotis paradoxa* and *Hedyotis alata* of Rubiaceace have been done for the first time and the chromosome number of both the species are also reported for the first time.

The genus *Hedyotis* shows a wide range of chromosome number varying from $n=11$ to $n=36$ indicating the genus is polybasic. Lewis (1962) suggested that $x=12$ is the primary base number of the genus. From the available records of chromosome number Rubiaceae (Fedorov 1974, Kumar & Subramaniam 1986) it appears that the $n=11$ is the most commonly recorded basic chromosome number. Lewis (1980) and Bedi et. al. (1981) were also of the opinion that $x=11$ is the basic chromosome number for the family. In the present study base number $x=13$ and $x=15$ have been recorded. If the genus is having $n=12$ as the primary base number then $n=13$ and $n=15$ have evolved by aneuploidy. Meiotic analysis is indicating that the cytomixis was noticed in 38 percent PMC and due to cytomixis additional mass of chromosomes as well as additional number of chromosomes were also recor-

ded. According to Thakur (1978) and Sinha (1985) cytomixis is also responsible for formation of aneuploids. However, Bir and Chatha (1987) could not observe cytomixis in the species *Hedyotis articularia* R. Br. (S) rather they observed this phenomenon in some other genus of the same family. They have supported the view that the polybasic nature of the genus may be due to aneuploids. But they were of the opinion that aneuploids might have originated as a result of hybridization involving polyploid taxa as 25.33 percent of the cytologically known taxa are at various polyploid levels in the genus.

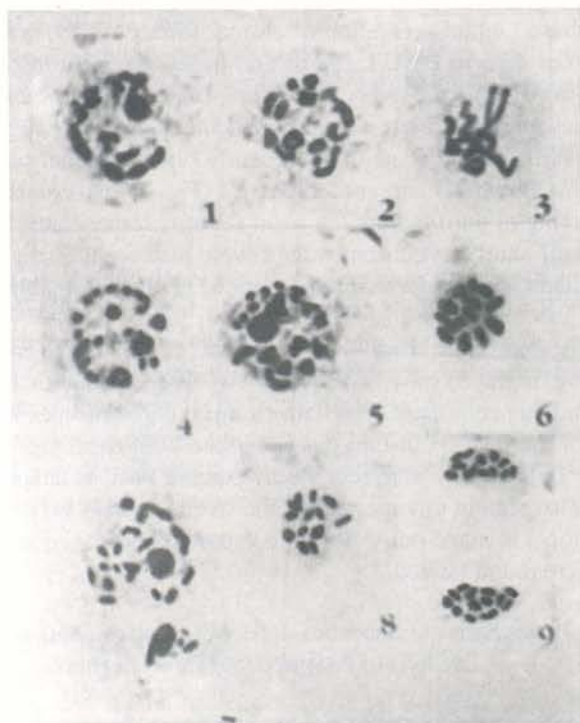


Plate I *Hedyotis* sps.

Fig. 1-5 and 7 - *H. paradoxa*.

- 1 and 2) PMC at metaphase I showing 15 II x 1500
- 3) PMC at diakinesis showing reciprocal translocation.
- 4 and 5) PMC at metaphase I showing univalents and multivalents x 1500.
- 7) PMC at diakinesis showing an additional patch of chromosome due to cytomixis.

Fig. 6, 8 and 9 - *H. alata*

- 6 and 8) PMC at metaphase I showing 13 II x 1500.
- 9) PMC at anaphase I showing equal distribution of chromosomes at the pole (13:13) x 1500.

Presence of multivalents may be attributed to the partial homology of the chromosomes but the possibility of translocation cannot be ruled out. Formation of diads, triads and polyads may be due to unequal separation of chromosomes at anaphase I and anaphase II which may be the result of spindle abnormalities. Such types of anomalies due to spindle abnormalities have also been reported by Thompson (1962).

Sterility of pollen grain is much more in *H. paradoxa* than in *H. alata*. This may be due to more percentage of cytomixis. In addition to cytomixis other abnormalities mentioned above are also responsible for sterility in both the species.

ACKNOWLEDGEMENT

Authors are thankful to Ministry of Environment and Forest for their financial help through Research Project.

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NEW CHROMOSOME COUNTS OF AN ENDEMIC SPECIES OF ANDAMANS

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ABSTRACT

Chromosome counts of *Bombax insigne* Wall. Var. *andamanica*. Prain. (Syn: *Salmalia insigne* (Wall) Schott & Endle Var. *andamanica* Prain) (*Bombacaceae*) an endemic species of andamans is reported for the first time. The detailed Cytology of the taxa has also been studied and discussed.

INTRODUCTION

Bombacaceae Kunth is a comparatively very small family of dicotyledons consisting of only 20 genera and one hundred eighty species. Only few species of the family have been studied cytologically so far. From Andamans and Nicobar islands only one species and three varieties of the genus *Bombax* Linn. have been recorded. Out of three varieties of *Bombax* recorded from Andamans two are endemic to Andamans. Among two endemic varieties recorded from Andamans specimen of only one variety *Bombax insigne* Wall. Var. *andamanica* Prain. has been examined (Rao 1986).

Bombax insigne Var. *andamanica* is very interesting as well as economically important plant. The plant is also having some medicinal value as some ayurvedic medicines are prepared from this plant. The fruits of the plant are eaten by Onge an endemic primitive tribe of andamans. Bhargava (1981).

Because of commercial exploitation of the variety in large scale the variety is threatened today. If immediate measures are not taken the germplasm of this important plant may vanish in near future.

Cytologically the taxa has not been examined so far. The present study has been undertaken with a view to preserve the germplasm of this threatened endemic plant. In the first phase of study the plant is examined cytologically and the Chromosome number and detailed Cytology of the plant is reported here for the first time.

MATERIALS AND METHODS

Flower buds are having very thick sepals which tightly covered the anther. Therefore before putting buds in saturated aqueous solution of para-dichlorobenzene the sepals were removed. After 24 hours buds were washed thoroughly in fresh water then fixed in Carnoy's solution. A few drops of ferric chloride were added in the fixative as a mordant to obtain good staining of the Chromosomes. After 24 hours buds were presented in 95 percent alcohol to avoid excess staining of cytoplasm.

Chromosome studies were made by squashing young anthers in 2 percent acetocarmine and microphotography was done from temporary slides.

Pollen grains were analysed by squashing mature anthers in 2 percent acetocarmine. Stained pollen grains were scored fertile and unstained as sterile.

Voucher specimens have been deposited in the herbarium of the department of Botany, J. N. R. Mahavidyalaya, Port Blair (Andamans).

RESULTS

Bombax insigne Wall. Var. *andamanica* Prain is large deciduous tree and an endemic plant of Andamans mostly found in South Andamans. Once the taxa was abundant in South Andaman. Natural regeneration of the species was abundant in the andamans especially in the areas where the shrubs were cut and burnt. In the recent years it has been observed that the population has reduced very much and now only few trees were found scattered in the South Andamans. The natural regeneration capacity has also reduced to a greater extent. Stem and brachlets are armed with conical prickles. Leaves digitate 5-9 foliate borne on a long common petiole measuring around 1 feet.

Leaves 5" to 8" long, elliptic obovate to oblanceolate. Flowers 3" to 6" long solitary, calyx 1" to 1½" long, silky inside, Petals red, stamen numerous, capsule 6" to 8" long. The tree start flowering at the age of 8 years and bears fruits in every alternate year.

For the present study flower buds were collected from Carbyn's Cove in December 1989, Collection No. 0292, Population 1289.

Chromosome number of the taxa $n=9$ is reported for the first time.

Meiotic division in general was found to be synchronized and normal. 95 percent pollen mother cells were seen with 9 bivalents at metaphase I (Fig. 1 and 2). Multivalents and univalents were seen only in few PMC.

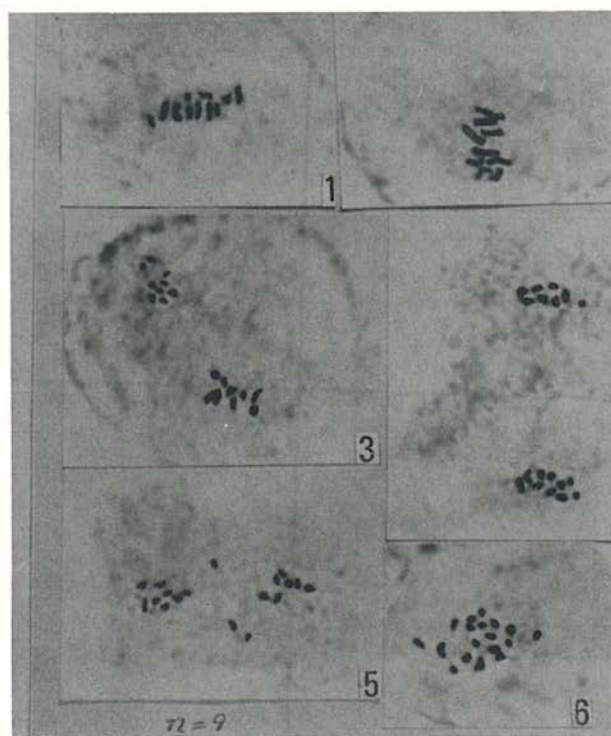
At anaphase I equal separation of chromosomes (9:9) were seen in most of the PMC (Fig. 3 and 4). Only in 5.6 percent PMC unequal separation of chromosomes were noticed (Fig. 5 to 6). Laggards were seen only in 4.8 percent PMC. Subsequent phases of division were found to be normal. Pollen sterility was found to be 21.2 percent.

DISCUSSION

The chromosome number of *Bombax insigne* Wall. Var. *andamanica* Prain. $n=9$ is reported for the first time. The chromosome number of the species is very interesting as the genetic number is lesser in comparison to the chromosome number of some other species of the same

same genus. *Bombax cieba* Lim. $2n=72$ Baker and Baker (1968), $2n=92$ Mehra and Sareen (1969) and $2n=96$ Sareen and Kumari (1973). *Bombax malabarica* Dc, $2n=72$ Janaki Ammal (1945). *Bombax brevicuspe* Sprange $2n=150$ Mangenot et. al. (1957) and *Bombax buonopozense* Beaur $2n=96$ Mangenot et. al. (1958). From the cited literature it appears that the other species of *Bombax* is at high level of polyploid. If. $n=9$ is considered as the prime base number than other species are at the tetraploid and pentaploid level, therefore polyploidy and aneuploidy might have played an important role in the evolution of these species. The plants of this polyploid complex showed certain recognisable morphological differences. In view of the morphological differences in association with the type of chromosomal difference, a detailed taxonomic study of this genus should be interesting.

Chromosome aberrations were few in the material studied in the form of laggards, bridges and unequal separation of chromosomes at anaphase I and II. This indicates that the species in question is cytologically and genetically stagnant and gradually losing re-generation capacity in the comparatively changed environment conditions of Andamans. To preserve the germplasm of the species it has become essential to activate the species genetically by inducing polyploidy, etc.



Bombax insigne var. *andamanica* $n=9$

Fig. 1 - 6

Fig. 1 - 2 PMC at Metaphase I showing 9 II x 1500.

Fig. 3 - 4 PMC at Anaphase I showing equal separation of chromosomes (9:9) x 1500.

Fig. 5 - 6 PMC at Anaphase I showing unequal separation.

ACKNOWLEDGEMENT

Authors are thankful to Ministry of Environment and forest, Govt. of India, New Delhi for financial help through Research project.

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A DERMATOGLYPHIC STUDY OF JORDANIAN POPULATIONS:

Part II: Palmar Configurations

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SUMMARY

Palm patterns of 970 phenotypically normal males (East-Bank: 480; West-Bank: 490) and 990 females (East-Bank: 492; West-Bank: 498) have been analysed. Results suggested that the incidence of palm patterns and their orders on the five interdigital areas (IVth > IIIrd > hypothenar > thenar/1st > IInd) seem to be the same in the two populations. Statistically sex differences were not significant for all the palmar configurational areas. In general, a comparison of the incidence of palm patterns on these five configurational areas failed to reveal any marked differences between East and West-Bank populations indicating that the two populations do not differentiate very clearly.

INTRODUCTION

The potential contribution of dermatoglyphic patterns in racial studies (Cummins and Midlo, 1926; Cummins, 1931; Steggerda *et al.*, 1936; Cummins and Setzler, 1951; Rife, 1954; Lestrangle, 1954 a, 1954 b; Kumbnani, 1959; Newman, 1960; Bhasin, 1971; Plato *et al.*, 1975; Mehdipour and Farhud, 1978, 1979; Sunderland and Denis, 1979; Kamali, 1981, 1982, 1984; Smit, 1987; Arrieta and Lostao, 1988; Stoney, 1988; Omari, 1991, 1992) and in medical disorder groups associated with chromosome abnormalities, with the ultimate purpose of early diagnosis and etiology (Alter, 1967; Penrose, 1969; David, 1971; Schumann and Alter, 1976; Kanematsu, 1982; Hirth *et al.*, 1985; Boerger, 1986; Rignell, 1987; Davee *et al.*, 1989; Otto, 1989) has long been appreciated. The present study extends these studies to a large sample of phenotypically normal subjects living in the East and West-

-Bank of Jordan. As no figures for Jordanians have been published before, this study is presented as a record of data toward an eventual analysis of racial variation, and as a means to evaluate dermatoglyphics of patients against those of a group of normal subjects of the same racial and geographical distribution.

MATERIALS AND METHODS

The material presented consists of palm prints of 970 males (East-Bank: 480; West-Bank: 490) and 990 females (East-Bank: 492; West-Bank: 498). None of the subjects studied had a diagnosed or suspected genetic or chronic disease of any kind. Only unrelated subjects were taken in this study. Each subject is represented with bilateral prints. The prints were collected and interpreted according to the method of Cummins and Midlo (1961). Dermatoglyphic features were evaluated and presented for each sex and each hand separately in order to investigate both the sex and bilateral differences. Bimanual and sexual comparisons have been carried out by means of contingency analysis.

RESULTS

The percentile frequencies of the true patterns and vestiges on the palmar configurational areas in both East- and West-Bank populations are given in Table 1. The two populations show similar frequencies of palm patterns. They are characterized by relatively high pattern frequencies in the hypothenar, third, and fourth interdigital areas and low frequencies in the thinar/first, and second interdigital areas. Patterns occur more frequently on left hands in the hypothenar and fourth interdigital areas, whereas they occur more frequently on right hands in the thinar/first, second, and third interdigital areas. These bimanual differences are evident in both males and females of East- and West-Bank populations. Statistically sex and population differences are not significant for all the palmar configurational areas (Table 2).

DISCUSSION

Patterns occur more frequently on right hands in the second and third interdigital areas, whereas they occur more frequently on the left hands in the hypothenar, thinar/first interdigital and fourth interdigital areas (Table 1). The same observation was made by Rife (1955) and Floris (1975). Cromwell and Rife (1942) compared the prints of the right and the left hands, and found a trend towards reduction of bimanual differences. In the present study, this trend was found to hold true for all areas except the hypothenar, where the bimanual differences were the highest of any of the areas. Attention may be directed to the differential bimanual occurrence of hypothenar patterns. The hypothenar patterns are more frequent in left hands, a condition which has been reported in several published studies (Cummins, 1935; Steggerda *et al.*, 1936; Rife, 1953; Bhasin, 1971; Qazi *et al.*, 1977; Rao *et al.*, 1983), in contrast to the typical "European" trend of a superiority of the right hand (Cummins and Midlo, 1927; Cummins and Shank, 1937; Viswanatham and Vijayalaksh-

TABLE I

**Incidence of Patterns (True Patterns and Pattern Vestiges)
in the Palmer Areas in East- and West-Bank Populations.**

Population	Sex	Hand	HYPO-THE- NAR	THINAR/ FIRST INTER- DIGITAL	SECOND INTER-DIGI- TAL	THIRD INTER-DIGI- TAL	FOURTH INTER-DIGI- TAL
East-Bank	M	R	36.10	13.06	9.60	48.90	54.16
		L	41.10	13.10	8.80	47.50	55.40
		R + L	38.60	13.08	9.20	48.20	54.78
	F	R	35.92	12.95	9.39	49.10	53.94
		L	40.93	13.01	9.01	48.01	55.10
		R + L	38.42	12.98	9.21	48.05	54.52
M + F	R	36.01	13.00	9.49	49.00	54.05	
	L	40.01	13.05	8.90	47.75	55.25	
	R + L	38.01	13.03	9.20	48.37	54.65	
West-Bank	M	R	35.98	12.90	10.00	50.10	53.80
		L	40.96	12.80	9.10	46.60	54.90
		R + L	38.47	12.85	9.55	48.35	54.35
	F	R	36.20	12.72	9.80	49.62	53.20
		L	41.00	12.94	9.02	47.02	55.10
		R + L	38.60	12.83	9.41	48.32	54.15
M + F	R	36.09	12.81	9.90	49.86	53.50	
	L	40.98	12.87	9.60	46.81	55.00	
	R + L	38.53	12.84	9.75	48.33	54.25	
East- and West-Bank	M	R	36.04	12.98	9.80	49.50	53.98
		L	41.03	12.95	8.95	47.05	55.15
		R + L	38.53	12.96	9.37	48.27	54.56
	F	R	36.06	12.83	9.59	49.36	53.57
		L	40.96	12.97	9.01	47.51	55.10
		R + L	38.51	12.90	9.30	48.43	54.33
M + F	R	36.05	12.90	9.69	49.43	53.77	
	L	40.49	12.96	9.25	47.28	55.12	
	R + L	38.27	12.93	9.47	48.35	54.44	

M = Male; F = Female; R = Right; L = Left.

TABLE II

Statistical Analysis of the Incidence of Patterns in the Palmar Areas

Population	Sex	Chi-Square Values*				
		HYPO-THE- NAR	THINAR/ FIRST INTER- DIGITAL	SECOND INTER-DIGI- TAL	THIRD INTER-DIGI- TAL	FOURTH INTER-DIGI- TAL
		East-Bank	M vs. F	0.4044	0.0000	0.0676
West-Bank	M vs. F	0.0016	0.0177	0.0053	0.0417	0.0299
East- and West-Bank	M vs. F	0.0003	0.0019	0.0417	0.0257	0.0075
E.-Bank vs. W.-Bank	M vs. M	0.0000	0.0077	0.0013	0.2068	0.0043
	F vs. F	0.0018	0.0020	0.0316	0.0577	0.0458
	M&F vs. M&F	0.0469	0.0000	0.0135	0.0734	0.1616

* = Statistically sex and intergroup differences are not significant for all the palmer configurational areas.

mi, 1980). Although the trends were similar in each of the other four areas, they were only of the magnitude of approximately 1 to 2%.

Considering the sex, the incidence of patterns on the five palmar areas was slightly higher in males than their female counterparts (Table 1), but statistically no significant differences between sexes were found within both East- and West-Bank populations (Table 2). In contrast to these findings, several studies showed sex differences in the incidence of patterns on one or more of these five palmar areas (Bhasin, 1971; Floris, 1976; Qazi *et al.*, 1977; Viswanatham and Vijayalakshmi, 1980; Rao *et al.*, 1983; Kamali and Sharif, 1985). These findings may suggest that East- and West-Bank populations stand entirely apart, a feature which may differentiate them from other populations.

A very close similarity in the incidence of patterns on the five palmar areas was observed among the East-Bank population and that from West-Bank population (Tables 1 and 2) indicating that the two populations are genetically indistinguishable with respect to the patterns of palmar areas. Omari (1986) has compared the frequencies of several blood group systems among East- and West-Bank populations and although certain differences were suggested, none were great enough to be significant. Omari also compared the fingerprint patterns (1985), taste reaction to phenylthiocarbamide (1986), mid-digital hair (1986), colour-blindness (1987), palmar main lines, modal types and main-line formulae (Omari, 1991), and main-line index and transversality (Omari, 1992), and no consistent and significant differences were apparent between East- and West-Bank populations. It seems reasonable at this point to assume that East- and West-Bank populations are essentially of the same ethnic stock.

By comparing the present figures with those obtained by others, it was noted that the incidence of palmar patterns agrees fairly closely with the previously reported incidences among Rwala Arabs and Syrians (Cummins and Shanklin, 1937).

The set of data presented in this study is a component of the physical anthropology of the general Jordanian populations. At the same time these data and others (Omari, 1985, 1991, 1992) can be used as controls when analysing the dermatoglyphic findings in Jordanian patients with genetic disease or congenital malformation.

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