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Isoenzymes of human lice: *Pediculus humanus* and *P. capitis*

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Abstract. Abstract. Human lice (Phthiraptera: Pediculidae) from Africa, America and Europe were electrophoresed for 28 enzymes, with special interest in metabolic factors likely to be involved with insecticide resistance. Zymogram profiles of the body louse (*Pediculus humanus* L. from France and U.S.A.) and the head louse (*P. capitis* DeGeer from France, Madagascar, Mali & Senegal) were compared. Only two enzymes, phosphoglucomutase (Pgm) and esterase 3 (Est-3), showed electrophoretic variation. In our starch gel electrophoresis conditions, *P. humanus* showed three electromorphs of Pgm migrating anodally 6, 11 and 16 mm (designated alleles *a*, *b*, *c*, respectively). Of the putative *Pgm* alleles, *b* and *c* occurred in all samples of both species of lice, whereas allele *a* was found only in *P. humanus* lab strain from U.S.A. Esterase 3 had four electromorphs migrating 23, 26, 30 and 35 mm (designated alleles *a*, *b*, *c* and *d*). Among putative *Est* alleles, *a* was found only in *P. capitis* from Bamako (all 14 specimens *aa* homozygotes), allele *d* was found only in *P. capitis* from Dakar (39% frequency), whereas *Est-3* alleles *b* and *c* showed apparently balanced polymorphism in all samples of both *P. humanus* and *P. capitis* except that from Bamako. Despite the limited amount of isoenzyme variation detected (only 2/31 polymorphic loci), divergences of *Est-3* and *Pgm* among *Pediculus* populations may be relevant to their biosystematics and resistance.

Key words. *Pediculus capitis, P. humanus*, electrophoresis, insecticide resistance, isoenzymes, lice, pediculicides, France, Madagascar, Mali, Senegal, U.S.A.

Introduction

Human lice are commonly regarded as indicators of personal hygiene. Body lice (*Pediculus humanus*) transmit epidemic diseases such as typhus and relapsing fevers, but head lice (*Pediculus capitis*) are not so medically important (Buxton, 1947; Fontan *et al.*, 1984; Robert, 1985; Gentilini, 1993). Historically, pediculosis probably affected all strata of all human cultures until modern hygiene practices and pediculicides have almost eliminated body lice from western society, despite the development of resistant strains, beginning with DDT-resistance in Japan (Kitaoka, 1952) and Korea (Hurlbut *et al.*, 1952). Since the 1970s, however, head lice infestation rates have risen – especially among schoolchildren – in many parts of the world (Gratz, 1997). This could be due to increased pupil density in schools, more social interactions in team sports and while playing, crowding in houses and transport allowing transfer of lice from one individual to another (dependent on hair length), or inadequate and inappropriate use of insecticides. The large increase of international trade that has taken place since the 1970s has resulted in more travel and population mixing, with increased dissemination and transmission of pediculosis (Bertholet, 1976). Many cases of *Pediculus* resistance to insecticides have been described (Wright & Pal, 1965; Clarck & Cole, 1967; Miller *et al.*, 1972; Moores *et al.*, 1994; Roberts & Andre, 1994; Siegfried & Zera, 1994; Whyard *et al.*, 1994; Mumcuoglu *et al.*, 1995; White

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& Walker, 1995; Gratz, 1997). Cross-resistance to pyrethroids is perhaps more likely in populations already resistant to DDT (Maunder, 1991), but this is not inevitable (Hemingway *et al.*, 1999). Resistance generally results from genetic changes affecting synthesis of enzymes such as acetyl-cholinesterase (Lamizana & Mouchet, 1976; Maunder, 1991; Poirié & Pasteur, 1991; Moores *et al.*, 1994; Whyard *et al.*, 1994), monooxygenase (Hemingway *et al.*, 1999), non-specific esterases (Siegfried & Zera, 1994) or transferases (Brogdon, 1989). In addition, pyrethroid resistance in *Pediculus* may be caused by nerve insensitivity giving so-called 'knockdown resistance' (Hemingway *et al.*, 1999).

Therapeutic trials of pediculicides and studies of *Pediculus* resistance to insecticides require thorough knowledge of the biology of lice and their metabolism which is governed by many enzymatic reactions. When using various strains of *Pediculus* for bioassays to assess the activity of pediculicides, the effects of these products may not be the same in strains of different origins (Chosidow *et al.*, 1994; Downs *et al.*, 2000). Variation within and between strains makes it desirable to screen multiple strains (from the laboratory and field isolates from different individuals, schools, cities, countries and continents) when evaluating candidate compounds and formulations such as pediculicides. The susceptibility or resistance status of lice to insecticides depends on the species (body lice and head lice are generally apomictic: Busvine, 1978; White & Walker, 1995), the personal population (*P. capitis* from one person's head may not be as sensitive as those from somebody else's head) and the geographical strain (reflecting variable therapeutic pressure between communities in different cities, countries and continent) (Gratz, 1997).

To begin to characterize populations of human lice biochemically and genetically, we profiled the electrophoretic variation of 28 enzymes in samples of *P. capitis* collected in schools of tropical Africa, Madagascar and France, compared with *P. humanus* from a French hospital and a long-established laboratory strain from U.S.A. (Culpepper, 1948; Zeichner, 1999). Most of these enzymes appeared to be monomorphic and identical in both species of lice. However, electromorph variations observed for phosphoglucomutase and an esterase allowed us to evaluate differences between *Pediculus* species and strains.

Materials and methods

Reagents

α- and β-naphthyl-acetate, Fast blue RR were from Fluka, Buchs, Switzerland. All other products were obtained from Sigma Chemical Co. (St Louis, MO, U.S.A.).

Lice

Pediculus capitis were collected from schoolchildren in and around Tours and Besançon in France, from Antananarivo in Madagascar and from two tropical African cities: Bamako in Mali and Dakar in Senegal. Live head lice were obtained using a fine-toothed anti-louse comb (Laboratoire ITAX de P. Fabre, Santé, France) or by manual delousing. To eliminate all host blood enzymes, the lice were kept isolated for 48 h, then deep-frozen in liquid nitrogen for transport.

Pediculus humanus were collected from the clothes of homeless people treated by the Emergency Service of the Centre Hospitalier Régional, Universitaire de Tours, France. The lice were transported in plastic boxes to the laboratory, where they were deep-frozen in liquid nitrogen. A substrain of the original U.S.A. colony of *P. humanus* (Culpepper, 1948) was obtained from LIN/ORSTOM (now Institute for Research & Development), Montpellier, and maintained in our Laboratoire de Parasitologie, Mycologie et Médecine Tropicale, Faculté de Médecine de Tours, France. Maintenance procedure involved placing the lice (≈ 100/batch) on a piece of cotton cloth for support (1 × 1 cm), kept in plastic boxes at 28°C and 70% relative humidity (Alpatov, 1942; Flemings & Ludwig, 1964). They were fed daily by placing the cotton piece on the shaved abdomen of a rabbit (Valade, 1986). Eggs were laid on the piece of cloth, to which they were stuck by an agglutinant substance produced by the maternal annex glands (Schmidt, 1938, 1939).

Electrophoresis

This was undertaken with 14% starch gels according to the procedure of Second & Trouslot (1980). Gel buffer: 0.005 M histidine HCl, pH 9.5, containing 0.0025 M NaCl; migration buffer: 0.41 M sodium citrate, pH 8.0. Each louse was squashed individually on a 6-mm diameter disc of Whatman N°1 filter paper. The paper disc was placed on the lower edge of the gel and the electrophoresis was run at 220 V, 25 mA for 4 h. Gels were then cut horizontally into slices of 3 mm thickness and each slice was used to assay one enzyme.

Enzyme assays

These followed the procedures of Pasteur *et al.* (1987). Esterases (EC 3.1.1) were stained with Fast blue RR, alpha and beta naphthyl-acetate dissolved in 0.1 M Tris-HCl buffer, pH 7.1 (Selander *et al.*, 1986; Gilot & Andre, 1995). Each slice of gel was incubated in the dark with 0.0016 M 1-naphthyl acetate, 0.0016 M 2-naphthyl acetate in 2% acetone in water and fast blue RR (0.1% w/v) at 37°C for 3 h.

Phosphoglucomutase (EC 5.4.2.2) was assayed with nicotinamide-adenine dinucleotide (Sutherland, 1949; Rathaur *et al.*, 1982); NAD 0.0004 M was dissolved in 0.1 M Tris-HCl, pH 8.5; phenazine methosulphate (PMS 6.5×10^{-6} M) and nitro-blue tetrazolium (NBT 250×10^{-6} M) were dissolved in 0.27 M sodium glutamate buffer, pH 7.0. The enzymes were identified by their activities and their relative migrations from the origin.

Table 1. Enzyme assays with *Pediculus* adults (apparent genetic polymorphism of esterase 3 and phosphoglucomutase represented by italics). Interference + or – with enzymes from rabbit blood, except E = interference between esterases and carbonic anhydrase.

Number	Enzyme name	Abreviation	E. C. Number	Interference	Migration	Characteristic
1	Lactate dehydrogenase	LDH	1.1.1.27	+	Anodal	Invariable
2	Malate dehydrogenase	ME	1.1.1.40	+	Anodal	Invariable
3	Peroxidase	POX	1.11.1.1	+	Cathodal	Invariable
4	Peptidase	PEP	3.4.11.4	+	Anodal	Invariable
5	Alkaline phosphatase	AKP	3.1.3.1	+	Anodal	Invariable
6	Aconitate hydratase	ACON	4.2.1.3	+	Anodal	Invariable
7	Adenylate kinase	AK	2.7.4.3	+	Anodal	Invariable
8	Glucose-6-phosphate 1-dehydro-genase	Gd	1.1.1.49	+	Anodal	Invariable
9	6-phosphogluconate 2-dehydro-genase	PGD	1.1.1.43	+	Anodal	Invariable
10	Superoxide dismutase	SOD	1.15.1.1	–	Anodal	Invariable
11	Phosphoglucoisomerase	PGI	5.3.1.9	–	Anodal	Invariable
12	Malate dehydrogenase	MDH	1.1.1.37	–	Anodal	Invariable
13	Glutamate dehydrogenase	GDH	1.4.1.2	–	Anodal	Invariable
14	Aspartate aminotransferase	AAT	2.6.1.1	–	Anodal	Invariable
15	Isocitrate dehydrogenase	ICD	1.1.1.42	–	Anodal	Invariable
16	Leucyl aminopeptidase	LAP	3.4.11.1	–	Anodal	Invariable
17	Acid phosphatase	ACP	3.1.3.2	–	Anodal	Invariable
18	Carbonate dehydratase	CD	4.2.1.1	E	Anodal	Invariable
19	Octanol dehydrogenase	OCT	1.1.1.73	–	Anodal	Invariable
20	Glycerol 3-phosphate dehydro-genase	GPD	1.1.1.8	–	Anodal	Invariable
21	Catalase	CAT	1.11.1.6	–	Anodal	Invariable
22	Glucokinase	GK	2.7.1.2	–	Anodal	Invariable
23	Pyruvate kinase	PK	2.7.1.40	–	Anodal	Invariable
24	Hexokinase	HK	2.7.1.1	–	Anodal	Invariable
25	3-hydroxybutyrate dehydrogenase	3-HBDH	1.1.1.30	–	Anodal	Invariable
26	Creatine kinase	CK	2.7.3.2	–	Anodal	Invariable
27	Phosphoglucomutase	PGM	5.4.2.2	–	Anodal	Variable
28a	Esterase 1	EST1	3.1.1	–	Anodal	Invariable
28b	Esterase 2	EST2	3.1.1	–	Anodal	Invariable
28c	Esterase 3	EST3	3.1.1	–	Anodal	Variable
28d	Esterase 4	EST4	3.1.1	–	Anodal	Invariable

Esterase inhibition effects

These were assessed in response to three inhibitors: eserine (physostigmine), malathion and ethylenediamine tetra-acetic acid (EDTA). Each inhibitor was dissolved in 1% acetone and added to the substrate solution to give final concentrations of malathion 0.0034 M, EDTA 0.0038 M and eserine 0.004 M.

Results

Selection of marker enzymes

Table 1 lists the 28 enzymes investigated in *Pediculus*. Those listed as 1–9 were discarded because of possible interference from rabbit blood, as detected by tests on *P. humanus* laboratory strain. Those listed as 10–26 showed no variation within or between strains and species, i.e. they were electrophoretically monomorphic. Only phosphoglucomutase (27) and esterases (28a–d) showed sufficient electrophoretic variability reflecting genetic polymorphism.

Esterases

Esterase activity profiles segregated as four bands migrating 17, 20, 23–35 and 55 mm during 3 h electrophoresis, designated as esterases 1, 2, 3 and 4 (28–d in Table 1). These esterases were differentiated by means of specific inhibitors (Table 2). Esterase 1 was slightly inhibited by malathion and EDTA but not by eserine. Esterase 2 was inhibited by EDTA only. Esterase 3 was inhibited by malathion and eserine, but not by EDTA, and esterase 4 was slightly inhibited only by EDTA. Esterases 1, 2 and 4 were invariable in all strains of lice tested. Esterase 3 (28c) behaved as a cholinesterase and was included because of its variability and because the rabbit blood esterases caused no interference (Table 1).

The esterase 3 zymogram (Fig. 1) showed a locus with four anodal electromorphs at 23, 26, 30 and 35 mm, designated alleles *a*, *b*, *c* and *d*, respectively (Table 3). *Pediculus humanus* carried only electromorphs 26 and 30 (alleles *b* and *c*). The wild sample of four specimens from France (genotypes 2*bb* and 2*cc*) was insufficient for statistically satisfactory comparison

with the laboratory strain (allele frequencies 0.67*b*, 0.33*c*), but they clearly shared the same *bc* polymorphism of *Est-3*.

Pediculus capitis from Senegal (Dakar, $n=14$) was the most polymorphic for Est-3 electromorphs 26, 30 and a fourth band at 35 mm (genotypes *bb*, *bc*, *bd* and *dd*). The whole sample of *P. capitis* from Mali (Bamako, $n=14$) was monomorphic for Est-3 electromorph 23 (genotype *aa*), but this band was not found in any of the other samples. Allele *d* (electromorph 35) was not seen in *P. capitis* from Madagascar ($n=4$), Mali ($n=14$) and France (Besançon, $n=4$; Tours $n=18$), nor in *P. humanus* (Tours, $n=4$; lab strain, $n=11$). Frequencies of the four putative alleles of esterase 3 are summarized in Table 3, showing apparently balanced *bc* polymorphism in *P. humanus* (France and U.S.A.), while *P. capitis* has apparently the same balanced polymorphism of *bc* (France and Madagascar), but also *bcd* (Dakar) and the anomalous Bamako population homozygous for *aa* genotype of *Est-3*.

Phosphoglucomutase

The phosphoglucomutase zymogram (Fig. 2) showed a locus with three anodal electromorphs at 6, 11 and 16 mm, designated alleles *a*, *b* and *c*, respectively (Table 3). *Pediculus humanus* carried balanced *abc* polymorphism (laboratory strain: 0.24*aa*:0.42*bb*:0.34*cc*), but only *bc* polymorphism was detected in the small sample from France (Tours: 1*bb*:1*bc*:2*cc*).

Pediculus capitis from France (Tours, $n=27$) carried *abc* polymorphism of *Pgm* (0.074*aa*:0.352*bb*:0.574*cc*), whereas the samples from Mali (Bamako, $n=8$) and Senegal (Dakar, $n=8$) apparently lacked Pgm^a but showed balanced *bc* polymorphism. Only one specimen of *P. capitis* from Madagascar was profiled for *Pgm* and showed homozygous electromorph 16 (genotype *cc*). Frequencies of the three putative alleles of phosphoglucomutase are summarized in Table 3, showing *Pgm* allele *a to* be rarest in both *P. capitis* (mean 4.2%) and *P. humanus*, despite reaching the highest frequency in the latter laboratory strain (24%). *Pgm* alleles *b* and *c* showed similar overall frequencies in both head and body lice.

Table 2. Differentiation of four esterases from *Pediculus* by three inhibitors: –, no inhibition; +, total inhibition; ±, decreased activity.

	Esterases			
Inhibitor	EST1	EST2	EST3	EST4
Malathion	±	–	+	–
Eserine	–	–	+	–
EDTA	±	+	–	±

Discussion

Despite their morphological similarity, the biology and medical importance of head and body lice are completely different (Buxton, 1947; Bertholet, 1976; Fontan *et al.*, 1984; Valade, 1986; Gratz, 1997). Because they occupy separate niches on the human body, they seldom interbreed (Busvine, 1978) and their insecticide resistance profiles tend to be distinct (White & Walker, 1995; Gratz, 1997). The head louse *P. capitis* is an obligate human parasite that cannot be maintained for long in the laboratory because it does not feed on rabbits or other mammals and requires multiple, frequent bloodmeals daily. The body louse *P. humanus* lives mainly off the human body, in clothes or bedding (Alliot & Combre, 1977); it can feed on rabbit blood, which is relatively well tolerated (Flemings & Ludwig, 1964) and may survive on only

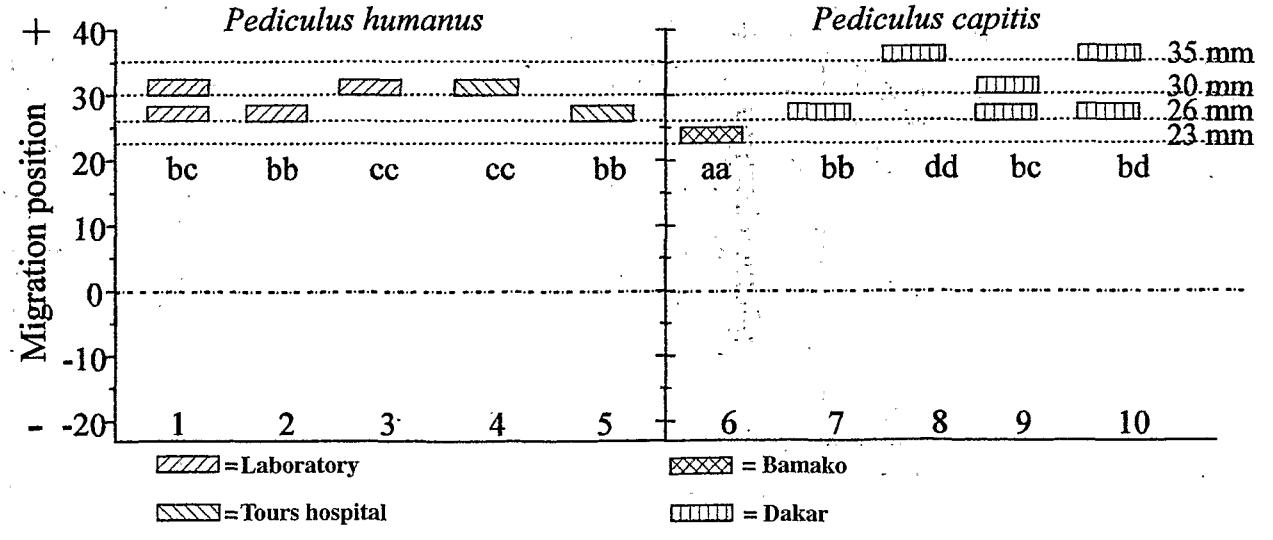

Fig. 1. Schematic representation of esterase 3 zymogram showing genotypes *aa*, *bb*, *bc*, *cc*. Lane numbers represent *P. humanus* (laboratory (1–3), Tours hospital (4–5)), *P. capitatis* (Bamako (6), Dakar (7–10)).

one bloodmeal per day. This makes it possible to maintain *P. humanus* as a laboratory strain.

As several enzymes have been implicated in resistance to insecticides (Brogdon, 1989; Maunder, 1991; Siegfried & Zera, 1994; WHO, 1998), we investigated the variability of enzymes from several populations of human lice. Electrophoretic analysis of 28 enzymes from various strains of body and head lice identified two polymorphic loci: esterase 3 and phosphoglucomutase, both of general metabolic importance. Because esterase 3 was found to be inhibited by malathion and eserine, it was interpreted as a cholinesterase. Esterases, particularly acetylcholinesterase, are commonly involved in resistance to insecticides (Beach & Cordon-Rosales, 1989; Coats *et al.*, 1994; Mutero *et al.*, 1994; Siegfried & Zera, 1994; Vaughan *et al.*, 1995; WHO, 1998). Whereas we consider the inbred laboratory strain of *P. humanus* to be fully susceptible to all insecticides (Zeichner, 1999), our other samples of lice were not tested for insecticide sensitivity. It remains to be seen whether the type of cholinesterase variation we detected (*Est-3* polymorphism) in 4/5 populations of *Pediculus* might form the basis for selection of any resistance mechanism. Otherwise, so little is yet known about *Pediculus*

Table 3. Allele frequencies (see Figs for codes of alleles and electromorphs) of two polymorphic isoenzymes (esterase and phosphoglucomutase) in seven geographical samples of *Pediculus*.

		Esterase 3					Phosphoglucomutase			
			e'morph: 23	26	30	35		e'morph: 6	11	16
Species and origin of sample	Date coll.	*n*	allele: a	b	c	d	*n*	allele: a	b	c
P. humanus										
Laboratory strain	01/94	11	0.000	0.720	0.280	0.000	32	0.240	0.420	0.340
Tours, France	01/94	4	0.000	0.500	0.500	0.000	4	0.000	0.375	0.625
Total or mean	01/94	15	0.000	0.666	0.333	0.000	36	0.213	0.414	0.372
P. capitis										
Bamako, Mali	12/93	14	1.000	0.000	0.000	0.000	8	0.000	0.500	0.500
Dakar, Senegal	12/93	14	0.000	0.430	0.180	0.390	8	0.000	0.690	0.310
Antananarivo, Madagascar	09/93	4	0.000	0.500	0.500	0.000	1	0.000	0.000	1.000
AFRICA	09/93	32	0.438	0.250	0.141	0.172	17	0.000	0.559	0.441
Tours, France	01/94	18	0.000	0.722	0.278	0.000	27	0.074	0.352	0.574
Besançon, France	09/93	4	0.000	0.500	0.500	0.000	4	0.000	0.750	0.250
EUROPE	09/93	22	0.000	0.682	0.318	0.000	31	0.065	0.403	0.532
Total or mean	09/93	55	0.255	0.418	0.209	0.100	48	0.042	0.458	0.500

n = number of diploid specimens tested (allele frequencies based on two haploid genomes per specimen).

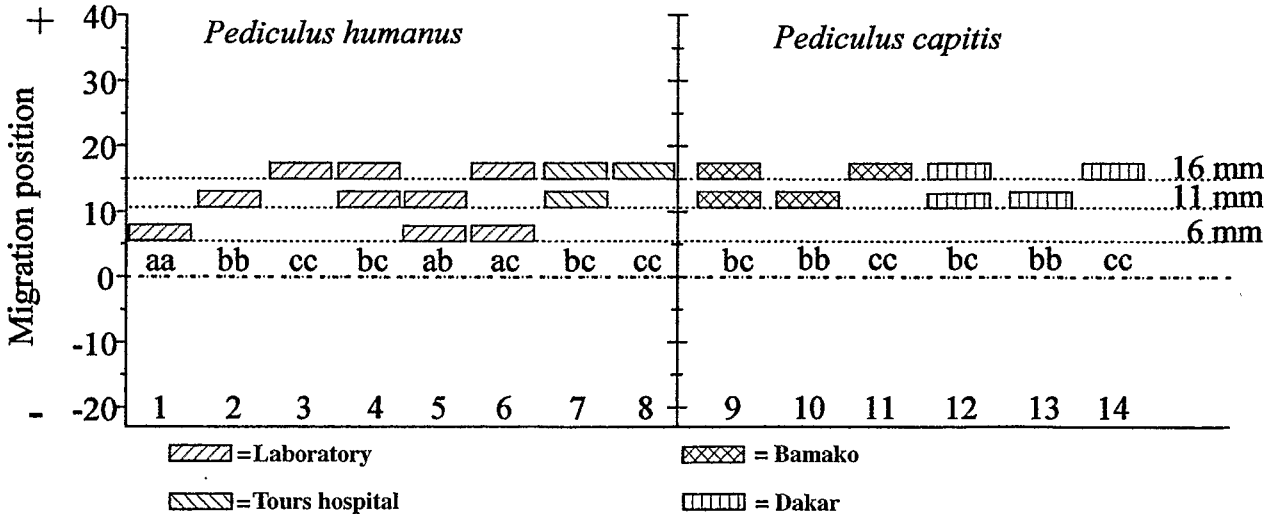

Fig. 2. Schematic representation of phosphoglucomutase zymogram showing genotypes *aa*, *ab*, *ac*, *bb*, *bc*, *cc*.
Lane numbers represent: *P. humanus* (laboratory (1–6), Tours hospital (7–8)), *P. capitatis* (Bamako (9–11), Dakar (12–14)).

biochemistry, despite efforts to immunize against human lice (Ben-Yakir *et al.*, 1994; Ochanda *et al.*, 1996).

Taxonomically these results show some signs of differentiation between head and body lice that should be further explored. Some African populations of *P. capitis* carried alleles *a* and *d* of esterase 3 (electromorphs 23 and 35) that were not detected in *P. humanus*. The cases of *Est-3*^{a} apparently fixed in *P. capitis* from Bamako, and detection of *Est-3*^{d} only in *P. capitis* from Dakar (39% frequency), are of special interest to investigate the local and wider significance in biosystematics of *Pediculus*. Are these geographical populations distinct, or are these alleles of esterase 3 more widespread? The unusually high frequency of phosphoglucomutase allele *a* in the laboratory strain of *P. humanus* might reflect genetic drift. Unfortunately, this electromorph is not diagnostic for *P. humanus*, as it was absent from the wild French sample but present at low frequency in 1/5 strains of *P. capitis*. The general lack of enzyme polymorphism at about 30 other enzyme loci (or our failure to detect any) proved to be a major limitation for further isoenzyme studies on the genetics, speciation and insecticide resistance potential of *Pediculus*.

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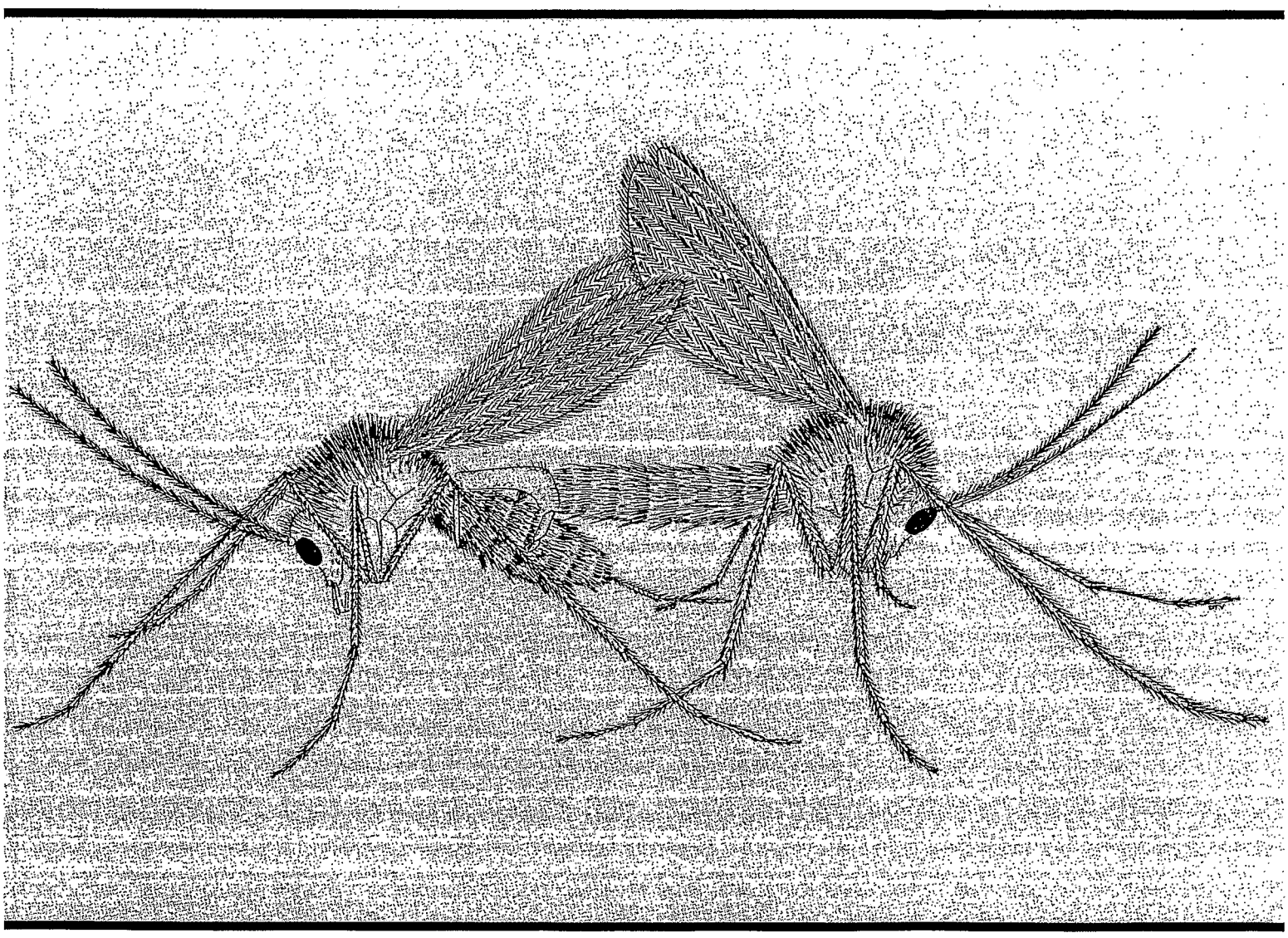

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