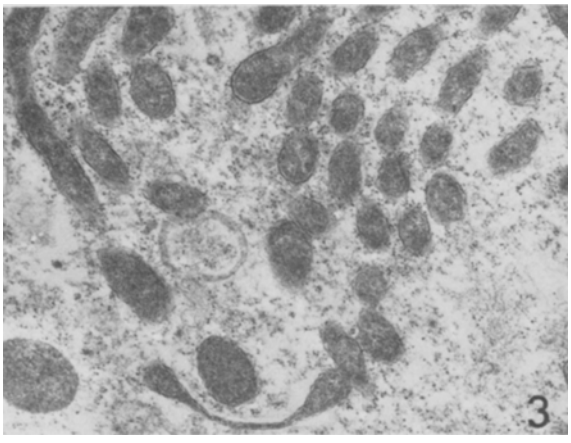

Fig. 3. Symbionts in the yolky region of young embryo. Note the absence of a host membrane. ×14,250.

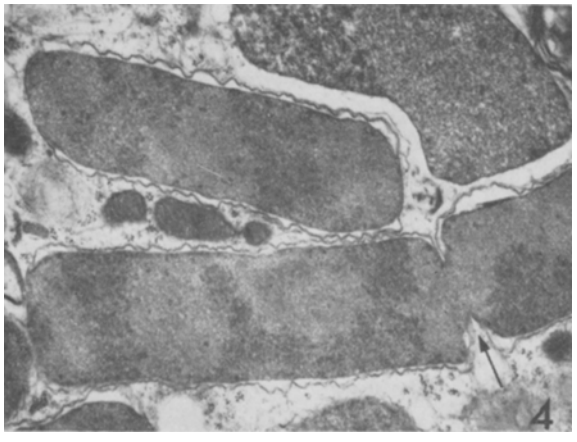

Fig. 4. Bacteroids in mid-gut mycetome. Arrow indicates characteristic division of Gram negative bacteria. ×14,500.

sects[12,13], certain of which have a similar reproductive system to tsetse flies, it has been shown that 2 symbiont types are definitely present, a gut form and a rickettsial ovarian form. Each of these is conveyed to the offspring along an independent pathway, the gut form moving in the milk secretion and the ovarian form via the egg. On present evidence we suggest that the rickettsia-like inclusions in this *Glossina* sp. are also passed from generation to generation in the egg, and that the Gram negative mid-gut symbionts, which from our and other[2] observations closely resemble the milk gland forms, are transmitted in the milk.

The precise role of bacterial symbionts within insect tissues has not been fully elucidated although there is evidence they contribute to the synthesis of factors essential for normal insect development[12]. Indeed, it has been demonstrated in some insect spp. that when the symbiont population is removed or reduced by antibiotics then growth is retarded and egg production is restricted[14]. Some recent experiments involving the antibiotic treatment of tsetse have also provided evidence for a considerable reduction of bacteroids in the mycetome[15]. Although it was not known at this time that ovaries carry symbionts it is interesting to record that the authors report degeneration of egg follicles and germaria. Similarly, female *G. austeni* fed upon rabbits whose diet contained a coccidiostat, sulphaquinoxaline and pyrimethamine, suffered marked interference in their ability to produce offspring, although it failed to increase the mortality rate of the mothers[16]. Although we have not examined females treated in this way one is nevertheless tempted to suggest that the rickettsia-like population could also be significantly reduced by the chemicals, in which case their presence may be essential for normal ovarian development. It should, however, be pointed out that this conflicts with the suggestion[7] that the rickettsia-like inclusions are to some extent parasitic and appear to cause degeneration of the tissues. We could detect no ill-effects in the host tissues and, moreover, the colony from which our material was drawn had normal fecundity.

As *Glossina* spp. are the vectors of the African trypanosomiases, our observations may have some bearing on the problem of insect control. It has been described how under certain conditions tsetses are virtually dependent on one species of domestic animal for their nutrition. The results outlined in this article serve to underline the suggestion[16] that by suitable host treatment a means of localized tsetse eradication may be achieved.

Summary. A rickettsia-like symbiont, located in the ovaries of *G. m. morsitans* is maternally transmitted to the offspring via the egg. It is suggested that they may be essential for normal ovarian development.

Résumé. Un symbionte de type rickettsien localisé dans les ovaires de *G. m. morsitans* est transmis maternellement par l'œuf à la progéniture. Le symbionte est probablement indispensable au développement normal des ovaires.

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Hybridization of Incompatible Poplars Following Solvent Treatment of Stigmas

Interspecific incompatibility barriers, normally preventing hybridization between white poplars and black poplars, are now well known. It was reported recently[1,2] that successful hybridization between *Populus deltoides*, from Section Aigeiros[3], the black poplars, and *P. alba*, from Section Leuce[3], the white poplars, could be accomplished experimentally using recognition pollen, i.e. compatible pollen rendered inviable by various means, which was then mixed with incompatible pollen and dusted on to receptive stigmas. Success rates of 15–30% seed set (5–11 fertile seeds per capsule, as compared with

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35 seeds per capsule for a compatible pollination) were obtained.

In discussing their results, the authors of these reports[1,2] emphasized the involvement of pollen wall proteins in the recognition process. They suggested that proteins liberated from the walls of killed compatible pollen were 'recognized', thus pre-disposing the stigma to accept the incompatible pollen mixed with it. The term 'recognition' is not a precise one in this context since it allows everything from self-not self immunological reactions, through enzyme-substrate interactions, to promoter-inhibitor balancing. It would certainly seem plausible ,if not essential, that a maternally-derived substance located on the stigmatic surface might also be involved in recognition. This would be in keeping with suggestions made[4,5] for the self-incompatibility (SI) mechanism in crucifers where cuticular lipids have been implicated, thus supporting an earlier suggestion[6] that the stigmatic cuticle is the important barrier in SI systems.

The present study sought to compare results from attempted white poplar/black poplar crosses using recognition pollen with others where stigmas were simply pretreated with lipid solvents before any attempted incompatible pollination.

In the first experiment, the black poplar female parent was a hybrid (*P. deltoides* × *P. fremontii*) while the incompatible white poplar pollen was derived from *P. alba* cv. 'Maktar'. Comparisons were possible between results of pollination after no pretreatment of stigmas, after pretreatment by careful wiping of stigmatic surfaces with a small brush very lightly moistened with hexane for a few seconds, and treatment with simultaneous addition of recognition pollen (Table I). It is seen that no successful crosses were made with incompatible pollen alone. The addition of recognition pollen allowed the production of about 3 fertile seed per capsule. In contrast, the pretreatment with hexane promoted, on average, about 18 fertile seeds per capsule which compares very favourably with an average of 19.4 for the control cross with the compatible Chilean Semi-evergreen, a form of *P. nigra* cv. 'italica'[7].

In the second experiment, the female parent was *P. deltoides* (provenance 'Mississippi'), the incompatible pollen was derived from *P. tremuloides*, and the compatible pollen from *P. nigra* cv. 'italica'. The treatments were the same as for the previous experiment, except that an ether pretreatment of the stigma was used in addition to the hexane pretreatment. Results (Table II) were even more striking than those obtained in the first experiment. Pretreatment with hexane enabled normally incompatible crosses to be made with a success rate of better than 97% of that of an acknowledged compatible one. Pretreatment with ether was only slightly less successful, enabling seed settings of over 95% of control values. The use of recognition pollen again resulted in successful setting of hybrid seed, but at a rate of just over 13% of control settings.

In both experiments, as in previous studies[1,2] subsequent sowings of the seed obtained, and analysis of seedling characteristics were used to verify the successful hybridizations resulting from the treatments.

These preliminary results focus attention once more on the role of the stigma in certain incompatibility mechanisms. It would seem likely that the hexane and ether pretreatments, which overcame innate incompatibility so completely, functioned to remove some substance(s) from the stigmatic surface. Furthermore, considering the solvents used, whatever is being removed might well be lipoid in nature, since treatment with cool or warm water and slight abrasion with the brush alone have never induced pollination. In extensions of this work[8], treatment of stigmas with dilute detergent also looks promizing, though quantitative results are not yet to hand. This lends further support to the notion that the incompatibility barrier in these cases might be in the form of emulsifiable lipids on the surface of the stigma.

Identification of the substance(s) removed from the stigmas by solvent treatment and the ascribing of definite roles to the pollen wall proteins are outstanding problems which must be solved before the operative incompatibility mechanism in vivo can be characterized. At the practical level however, the very simplicity of the solvent method and its demonstrated effectiveness in

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Table I. Numbers of fertile seeds per capsule (10 capsules per sample) from manipulated poplar crosses

♀ Parent – *P. deltoides* × *P. fremontii* hybrid			
(♂ Parent)			
P. alba cv. 'Maktar'			Semi-evergreen
+ Hexane	+ RP[a]	+ 0	+ 0
18	3	0	14
20	4	0	22
17	3	0	21
14	5	0	24
21	2	0	17
18	0	0	16
21	1	0	18
17	4	0	19
17	2	0	21
16	3	0	22
Mean 17.9	2.7	0	19.4

[a] Recognition pollen (see text).

Table II. Numbers of fertile seeds per capsule (10 capsules per sample) from manipulated poplar crosses

♀ Parent – *P. deltoides* 'Mississippi'				
(♂ Parent)				
P. tremuloides				*P. nigra* cv. 'italica'
+ Hexane	+ Ether	+ RP[a]	+ 0	+ 0
24	23	4	0	27
3	23	1	0	24
27	24	5	0	24
25	28	3	0	27
25	25	2	0	23
27	22	5	0	26
24	21	6	0	27
28	29	1	0	27
21	27	3	0	28
27	24	4	0	24
Mean 25.1	24.6	3.4	0	25.7

[a] Recognition pollen (see text).

promoting the intersectional hybridization of poplars, must prove a boon to breeders. Large numbers of hybrid progeny can be obtained, much greater than with recognition pollen, so that selections for cloning may be made from a much wider choice. This is very desirable since the percentage of valuable types produced by these wider crosses will always be smaller than for compatible crosses.

Summary. Pretreatment of stigmas of poplar flowers with hexane or ether promoted hybridization between normally incompatible black and white poplar species. Success rates, as measured by setting of fertile hybrid seed, exceeded 95% of those resulting normally from compatible crosses. Results suggest the existence of some incompatibility factor, possibly of a lipoid nature, located on the stigmatic surfaces.

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A23187 and Red Cells: Changes in Deformability, K^+, Mg^{2+}, Ca^{2+} and ATP

Incorporation of calcium into the interior of the red cell markedly reduces the deformability of the cell membrane[1,2]. Because calcium is efficiently pumped out of the normal red cell[3], experimental incorporation of calcium has required depletion of cellular ATP to allow leakage[1], or hemolysis and resealing[4]. The ionophore A23187 transports divalent cations across cell membranes[5–7], and we have used this ionophore to introduce calcium into red cells containing normal amounts of hemoglobin and ATP in order to investigate deformability in a system as intact as possible.

Materials and methods. Human blood was collected in ACD solution and immediately washed 3 times with Na-*tris* (135 m*M* NaCl; 4.5 m*M* KCl; 15 m*M* choline; 10 m*M* *tris*-HCl, pH 7.3) or K-*tris* (140 m*M* KCl; 15 m*M* NaCl; 10 m*M* *tris*-HCl, pH 7.3) buffer, supplemented with 1m*M* EDTA in the first wash to remove exogenous calcium[8]. Stock solutions (1 *M*) of the above monovalent salts were treated with Chelex resin (Dow) to remove Ca^{2+} and Mg^{2+}. Cells were resuspended to 10% hematocrit in the washing buffer and the divalent cation transport antibiotic A23187 (at 6 mg/ml in 75% ethanol: 25% DMSO) was added to make 0.1% of the volume for a final antibiotic concentration of 10 μm. (A23187 was a gift of Dr. R. L. HAMMILL of Eli Lilly and Co.[9].) After 30 min equilibration at room temperature divalent cations were added and incubated with the cells for 30 min.

Uptake of Ca^{2+} and Mg^{2+} was measured using atomic absorption spectroscopy. Intracellular Na and K contents were measured by flame photometry on cells washed 1 to 3 times in isotonic $MgCl_2$.

The deformability of the cells was measured by filtration through polycarbonate filters with 2.5 μm pores (Nuclepore, General Electric Corp), at 10 mm H_2O pressure, or by measurement of the pressure required to pull red cells through micropipettes as described previously[1]. ATP content was measured by a luciferin-luciferase method[10].

Results. The minimal solution concentration of A23187 required to produce the effects described below was 1 λ of stock A23187 solution per ml of red cells at 10% hematocrit. Upon addition of A23187, cells suspended in Na-*tris* medium shrank by 25%, while intracellular K^+ fell from 95 to 50 m*M* and intracellular Na^+ rose from 9.3 to 12–16 m*M* (Table I). The rapid K^+ efflux has been reported by others[6].

Addition of calcium to A23187-treated cells altered the mean cell volume (MCV) in proportion to the calcium added. The apparent shrinkage caused by 5 μ*M* Ca^{2+} correlates with increased filterability of these cells, but higher concentrations of calcium caused filterability to decrease markedly despite shrinkage. Addition of magnesium caused a similar loss of MCV but filterabtyili was unchanged. Thus MCV and K^+ content appear to be most strongly influenced by A23187, but cell filterability appears to be regulated primarily by calcium.

Deformability of cells was also measured by use of a micropipette technique[1] (Table II). Addition of A23187 alone slightly increased the pressure required to aspirate the cells, and also changed the morphology. Addition of calcium caused a marked increase in Pt for A23187 cells.

Since loss of intracellular ATP can cause loss of deformability, the ATP levels of cells containing A23187 and A23187 plus calcium were measured. The results, shown in Table III, indicate that addition of A23187 caused accelerated depletion of ATP relative to controls. However, addition of calcium did not produce further loss of ATP. Depletion of ATP thus appears to be caused by the A23187 itself. The mechanism of this rapid loss is not readily explained.

Table I. Changes in filterability, mean cell volume and monovalent cation content

Addition	Filterability (%)	MCV (μ³)	K_i	Na_i
None	59	91	95	9
A23187 only	59	73	50	13
A23187 + 5 μ*M* $CaCl_2$	71	62	42	—
A23187 + 50 μ*M* $CaCl_2$	46	67	33	16
A23187 + 500 μ*M* $CaCl_2$	10	72	35	—
A23187 + 50 μ*M* $MgCl_2$	61	68	37	19

Washed red cells at 10% hematocrit in Na-*tris* buffer were incubated 30 min with A23187 and an additional 30 min with divalent cations. Controls were incubated in parallel. Filterability was measured as described. Mean cell volume (MCV) in cubic microns; intracellular K and Na, in mmole/l packed cells. Concentrations of divalent cations are the amounts added to the cell suspension.

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