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Genetics of Gliding Motility in *Myxococcus xanthus* (Myxobacterales): Two Gene Systems Control Movement

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Summary. A large number of motility mutants of the gliding bacterium *Myxococcus xanthus* have been isolated and analyzed by transduction. Almost all nonmotile mutants are found to be double mutants. This is explained by the existence of two parallel and almost independent multi-gene systems controlling motility, in which case at least one mutation in each system would be required eliminate motility. Only one locus, called *mgl*, has been found to be shared by both systems. Wild type cells move singly and in groups. Single cells move if they carry a complete gene system A, the genes of which are described in the preceding paper. Groups of cells can move if they carry a complete gene system S, but single A^-S^+ cells do not move. Linkage analysis reveals at least 9 different loci in system S. One class of S^- mutants, those mutated in the locus *tgl*, are conditional mutants which, after contact with tgl^+ cells, become temporarily motile as cell groups. Most system A mutations have little effect on fruiting but many system S mutations block it, suggesting that system S plays a role in the fruiting process.

Introduction

The life cycle of *Myxococcus xanthus*, a fruiting myxobacterium, has two alternative pathways. When food is abundant, cells grow vegetatively with a generation time of 3 to 4 h. When food supplies are exhausted, *M. xanthus* fruits: thousands of cells come together to construct a haystack shaped fruiting body, a dense aggregate of cells within which individual cells transform into ovoid, desiccation-resistant myxospores (Dworkin, 1966). Cooperative movement is clearly necessary to construct a fruiting body since many cells must move into the same small area and pile on top one another. Cooperative movement also occurs during vegetative growth in the form of a vegatative swarm. Although swarm cells appear to move independently, they seldom leave the group and an entire swarm expands or migrates as a loose unit (Kühlwein and Reichenbach, 1968). How is movement coordinated in swarming and in fruiting?

Cells of wild type *M. xanthus* can move singly and in groups. The products of some 21 genes are necessary for the gliding of single cells (Hodgkin and Kaiser, 1979). Mutants in 5 of these genes, *cglB*, *cglC*, *cglD*, *cglE*, and *cglF*, can be complemented in cell mixtures. They are stimulated to move transiently by contact with other mutant or wild cells (Hodgkin and Kaiser, 1977). In this paper we describe the isolation and properties of mutants defective in the gliding of groups of cells. Movement of single cells and movement of groups of cells are resolved in various mutants in a way that leads us to propose that motility in *M. xanthus* is controlled by two multi-gene systems, one regulating the movement of single cells, the other regulating the movement of cell groups. Tests of this proposal are made and the roles played by the two kinds of movement in the process of fruiting are examined.

Methods

Strains. Table 1 lists the strains used, their origin, and their phenotypes. Evidence for some of the genotypes listed are given in Hodgkin and Kaiser (1977 and 1979); data for the rest are given in Results.

Nomenclature. Five gene names are used: *agl* (system A gliding); *cgl* (system A, conditional or contact stimulated gliding); *mgl* (system A and system S, mutual function for gliding); *sgl* (system S gliding); *tgl* (system S, conditional or contact stimulated gliding).

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able 1. Catalog of strains

train	Genotype	Motility phenotype[a]	Source		
)K100	*sglA*$^+$	AS	*M. xanthus* FB strain YS (Wireman and Dworkin, 1975)		
)K101	*sglA1*	A	Spontaneous mutant of FB		
)K323	*cglC1 sglA1*	N	UV on DK101		
)K342	*aglH2 sglA1*	N	UV on DK101		
)K1050	*sglA*$^+$	AS	A single colony isolate of FB		
)K1203	*cglC1*	S	UV on DK323		
)K1207	*cglB7 cglD1 sglA1*	N	UV on DK345		
)K1209	*cglC6 cglD1 sglA1*	N	UV on DK345		
)K1211	*aglR4*	S	Mx8 transduction ($S^- \rightarrow S^+$)	DK301	These strains were obtained from DK101 by mutagenesis and screening for non-motility (see accompanying paper, Hodgkin and Kaiser [1979])
)K1212	*aglJ1*	S		DK302	
)K1213	*aglN1*	S		DK305	
)K1214	*agl-12*	S		DK312	
)K1215	*aglA1*	S		DK316	
)K1216	*aglQ1*	S		DK319	
)K1217	*aglB1*	S		DK320	
)K1218	*cglB2*	S		DK321	
)K1219	*cglC1*	S		DK323	
)K1220	*aglF1*	S		DK326	
)K1221	*aglG1*	S		DK327	
)K1222	*aglE2*	S		DK329	
)K1225	*aglB3*	S		DK337	
)K1226	*aglP1*	S		DK338	
)K1227	*aglH2*	S		DK342	
)K1228	*aglC7*	S		DK343	
)K1229	*aglD2*	S		DK356	
)K1230	*cglE1*	S		DK360	
)K1234	*cglF1*	S		DK370	
)K1236	*aglR2*	S		DK390	
)K1237	*cglB7 cglD1*	S	Mx8 on	DK1207	
)K1238	*cglC6 cglD1*	S	Mx8 on	DK1209	
)K1243	*agl-12 sglA9*	N	UV on	DK1214	
)K1244	*aglA1 sgl-44*	N		DK1215	
)K1245	*aglG1 sgl-45*	N		DK1221	
)K1248	*aglB3 sgl-48*	N		DK1225	
)K1250	*aglB1 tgl-1*	N		DK1217	
)K1251	*aglJ1 tgl-2*	N		DK1212	
)K1252	*cglF1 tgl-3*	N		DK1234	
)K1253	*tgl-1*	A	Mx8 on	DK1250	
)K1256	*mgl-11 tgl-1*	N	UV on	DK1253	
)K1257	*mgl-10 cglE1*	N	UV on	DK1230	
)K1258	*sglB9*[b]	A	Mx8 on	NM	
)K1259	*aglB1 sglG1*	N	UV on	DK1217	
)K1260	*aglR4 sgl-60*	N	UV on	DK1211	
)K1261	*cglB2 sglC1*	N	UV on	DK1218	
)K1263	*cglB2 sgl-63*	N			
)K1264	*cglB2 sglD1*	N			
)K1265	*cglB2 sglA2*	N			
)K1266	*cglB2 sglB1*	N			
)K1272	*cglC1 sglE1*	N	UV on	DK1219	
)K1274	*cglC1 sglF1*	N	UV on	DK1219	
)K1283	*cglF1 sglA3*	N	UV on	DK1234	
)K1292	*aglR2 sglB3*	N	UV on	DK1236	
)K1300	*sglG1*	A	Mx8 on	DK1259	
M	*aglE3*	S	Burchard (1970)		
M	*aglE3 sglB9**	N	Burchard (1970)		
MM36-1c	See text	N	MacRae and McCurdy (1976)		
MM36-3d	See text	N	MacRae and McCurdy (1976)		
MM36-5L	See text	N	MacRae and McCurdy (1976)		
MM36-6J	See text	N	MacRae and McCurdy (1976)		
NS	*sglA10*	A	Wireman and Dworkin (1975)		
)Z2	*sglA*$^+$	AS	Campos and Zusman (1975)		

"A", short for A-motile, designates a phenotype in which single cells move. The right frame of Fig. 1 depicts A-motility. S", short for S-motile, designates a phenotype in which groups or rafts of cells move. The left frame of Fig. 1 depicts -motility. "AS", short for AS-motile, designates a phenotype in which both single cells and groups of cells move. An example is the upper left frame of Fig. 2. We do not know whether or not all strains with an AS-motile phenotype ave the same genotype. In fact these strains differ in the morphology of their colonies and in the rate at which the olonies expand. "N", for nonmotile, describes the phenotype in which cells do not move.

Strains mentioned in the text but not listed in this table may be found in Table 1 of the preceding paper

DK1258 and NM may carry a second *sgl* mutation; see Results for evidence

The following is intended as a glossary of terms to be used after or during the reading of Results. "System A" refers to the ensemble of genes that control A-motility. At present this includes *mgl* and all the *agl* and *cgl* loci. When a strain carries all the genes of system A in their wild type state, that strain is said to be "A^+". It is an experimental fact that all A^+ strains so far examined express A-motility, i.e. movement of single cells (Hodgkin and Kaiser, 1979). Analogously "system S" refers to the ensemble of genes that control S-motility. As described in *Results*, system S includes *mgl*, *tgl*, and all the *sgl* loci. When a strain carries all the genes of system S in their wild type state, that strain is said to be "S^+". All S^+ strains examined so far express S-motility, i.e. movement of groups of cells.

Media, *Mutant Isolation*, and *Motility Stimulation* have been previously described (Hodgkin and Kaiser, 1977, 1979). PM plates are 10 mM potassium phosphate pH 7.6, 8 mM $MgSO_4$, 1.5% agar.

Transduction and Selection of S-motile Bacteria. Reconstruction experiments (Table 2) showed that S-motile flares, as depicted in Fig. 1 of Results, can be detected when S-motile and nonmotile cells are mixed in a ratio as low as $1:10^6$.

Other experiments analogous to those reported in Hodgkin and Kaiser (1979) showed that the number of S-motile flares increases in proportion to the multiplicity of infection of transducing Mx8 at low multiplicity. UV treatment of the transducing lysate was found to increase the number of transductants without appreciably increasing the frequency of reversion or decreasing the frequency of cotransduction of *tgl* and *rif*.

A nonmotile double mutant of the type A^-S^-, when treated with phage grown on an A^+S^+ strain might be expected to give rise to A^+S^- and A^-S^+ transductants in approximately equal numbers. In practice (Table 3), A-motile flares are almost always more frequent than S-motile flares. Both the absolute and relative efficiencies of detection of the two types of flares are affected by the composition of the medium. For detection of S-motile flares we have adopted a solid medium consisting of 0.5% casitone, 0.1% yeast extract, 8 mM $MgSO_4$, 10 mM Tris at pH 7.6, 1.5% agar. Otherwise the transduction protocol was the same as described in Methods of Hodgkin and Kaiser (1979). Further attempts to improve the transduction assay are in progress.

Transduction and Selection of Rifampicin Resistance. Spontaneous Rif^R mutants are obtained at a frequency of about 2×10^{-8} after plating Rif^S *M. xanthus* strains on CTT agar containing 10 μg/ml of rifampicin (Calbiochem). Mx8 phage stocks grown on these strains were used for transduction without UV irradiation. Phage and recipient bacteria were mixed, usually at an infection multiplicity of 2, and shaken in CTT broth for 12–24 h at 33° C to allow expression of the recessive rifampicin resistance, before plating on selective media.

Table 2. Efficiency of detection of S-motile bacteria as flares

Bacteria added				Number of S-motile flares
Nomotile		S-motile		
No.	Strain	No.	Strain	
5×10^7	DK306	124	DK1203	25, 31
5×10^7	DK306	0		0, 0
5×10^7	DK307	45	DK1203	22
5×10^7	DK307	0		0
5×10^7	DK323	45	DK1203	31
5×10^7	DK323	0		0

The bacteria specified were mixed and the mixture treated as in the standard transduction protocol described in Methods of Hodgkin and Kaiser (1978). In the experiment with DK306 the spots were placed on CTT agar. In the experiment with DK307 and DK323 the spots were placed on 1/2 CTT agar.

Although DK1203 is capable of stimulating A-motility in DK307, spurious A-motile flares did not arise in these experiments, perhaps because stimulation is transient and there are very few DK1203 cells

Table 3. Effect of medium on detection of motile transductants

Agar	Other	S-motile flares: A-motile flares		
		Casitone 0.25%	0.5%	1%
1.00%	–		17:29	
1.25	–		13:29	
1.5	–	26:51	19:40	7:22
1.75	–		0:19	
2.00	–		0:17	0:11
1.5	5 mM Phosphate pH 7.6	10:46	15:44	5:34
1.5	2 mM $CaCl_2$	17:38	30:36	24:27
1.5	0.1% yeast extract	22:45	31:44	9:11
1.5	0.25% yeast extract	37:41	30:26	8:16

All media contained 10 mM Tris · HCl pH 7.6 and 8 mM $MgSO_4$. Recipient bacteria DK343 (*aglC7 sglA1*) at 4×10^7 cells per cross were mixed with Mx8 that had been grown on DK 100 (A^+S^+) at a multiplicity of 5. Apart from the medium, the standard transduction protocol was followed. Controls were carried out on medium that contained 1.5% agar, 0.1% yeast extract, 0.5% casitone, 8 mM $MgSO_4$ and 10 mM Tris pH 7.6. With no phage added, or with phage grown on DK343, there were no flares of either type; when the recipient bacteria were omitted there were no flares of either type

Results

1. Evidence for Genetic Control of Two Patterns of Cell Movement

Nonmotile mutants rarely arise from most strains of *M. xanthus* FB, e.g. strain DK100, but a spontaneous mutant of FB, called DK101, gives rise to many different nonmotile mutants (Hodgkin and Kaiser, 1979). If a nonmotile mutant derived from DK101 is exposed to a stock of transducing phage grown on DK100, then two different kinds of transduction flares are observed as shown in Fig. 1. One kind, shown in the left half of Fig. 1, has at its edge only groups or rafts of about 100 cells each. The other kind, shown in the right half of Fig. 1, has mostly single cells at its edge. Since these two phenotypes recur frequently in the material to follow, we designate them the "S-motile" phenotype (S for *social*

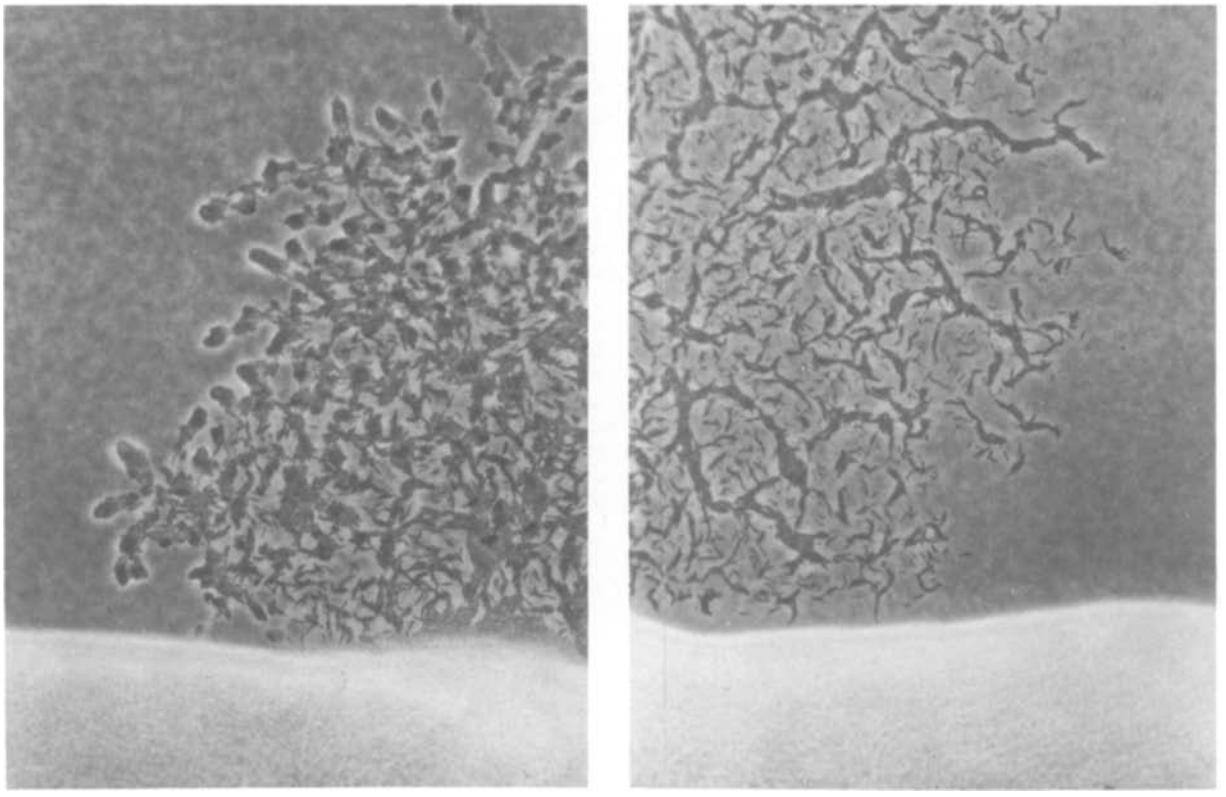

Fig. 1. Two types of flares observed after transduction of DK323 (*cglC1 sglA1*) with a transducing lysate grown on DK100: (right) A-motile, (left) S-motile. The smooth edge of the spot of DK323 from which the flares have arisen is visible near the bottom of each photograph

because groups predominate as for SM the first mutant of this type discovered by Burchard [1970]) and the "A-motile" phenotype (A for *a*dventurous because single cells predominate).

The A-motile flare in Fig. 1, which arose when DK323 was transduced with phage grown on DK100, resembles the colony morphology of DK101 itself (Fig. 1 in Hodgkin and Kaiser, 1978) and also resembles the flares produced when a nonmotile mutant derived from DK101 is transduced by phage grown on DK101 (Fig. 3 in Hodgkin and Kaiser, 1979). It therefore seems likely that the A-motile flare of Fig. 1 arose from the transduction of *cglC1* in DK323 to $cglC^+$. What is the origin of S-motile flares that arose in the same transduction experiment? DK101 differs from DK100, the transduction donor, in the frequency with which it gives rise to nonmotile mutants and, as will be shown below, in its pattern of cell movement. We suggest that DK101 carries a mutation responsible for its ability to give nonmotile mutants frequently and for its pattern of motility; and we call the relevant locus *sglA*. In these terms DK101 and DK323, which arose from DK101 by mutation, would be $sglA^-$ and the S-motile flares would arise the transduction of $sglA^- \rightarrow sglA^+$. A in *sglA* refers to the first locus of the *sgl* series; it carries no implication of A-motility.

This explanation is substantiated by the fact that both S-motile and A-motile flares arise when different nonmotile mutants derived from DK101 are transduced with phage grown on various *M. xanthus* FB derivatives (Table 4). Donors for the experiments of Table 4 were all originally derived from *M. xanthus* strain FB, but were propagated independently in three different laboratories for more than 5 years. The data show the frequent occurrence of S-motile flares and make it evident that $sglA^+$ is present not only in strain YS (Wireman and Dworkin, 1975), but also in strain NM (Burchard, 1970), in strain NMM36-5L (MacRae and McCurdy, 1976), and in strain DZ2 (Campos and Zusman, 1975). No S-motile flares arose when DK101 was the donor, as expected. About half of the collection of *agl* and *cgl* mutants derived from DK101 and described in the preceeding paper (Hodg-

Table 4. Transduction of the *sglA* locus

Recipient bacteria	Transductional donor							
	None	YS	DZ2	NM	NMM36-5L	YNS	NMM36-3d	DK101
DK323 (*cglC1 sglA1*)	0:0	10:36	4:14	3:21	20:50	1:34	0:49	0:28
DK343 (*aglC7 sglA1*)	0:0	29:44	12:29	11:35	41:50	0:40	0:48	0:38

Transduction was performed with 4×10^7 bacteria and Mx8 at a multiplicity of 5. Scores are presented as the number of spots with S-motile flares: the number of spots with A-motile flares. The maximum score of either is 55

kin and Kaiser, 1979) were exposed to transducing phage grown on DK100: all gave rise to S-motile flares. Therefore, the mutant locus *sglA* exists in all nonmotile strains derived from DK101. And, since transducing phage from most of the FB-derived strains produced S-motile transductants (Table 4), it is likely that the original FB (Dworkin, 1963) was $sglA^+$.

When used as transductional donors, strains YNS (Wireman and Dworkin, 1975) and NMM36-3d (MacRae and McCurdy, 1976) gave no S-motile flares with $sglA^-$ recipients (Table 4) and, therefore, like DK101 may carry mutant alleles of *sglA*. All the transductions reported in Table 4 also produced A-motile flares. Thus DK323 and DK343 behave like double mutants with one mutation in common, namely, *sglA*. They differ in their second mutation: *cglC1* in DK323 and *aglC7* in DK343 (Hodgkin and Kaiser, 1979).

S-motile transductants from these experiments were picked, purified, and examined. Among the S-motile strains obtained, all *agl* mutants ($sglA^+$ agl^-) were found to be similar in phenotype, as were their nonmotile *sglA agl* double mutant parents (Hodgkin and Kaiser, 1979). Most appeared to be more motile as measured by rate of colony spreading than SM (Burchard, 1970). However, some were less motile than SM, in particular $sglA^+$ $aglH^-$ and $sglA^+$ $aglM^-$. Instability that was apparent in some of the original nonmotile mutants, like that carrying *aglG1* (cf. Table 10 of the preceding paper) was also apparent in the colony phenotypes of their S-motile descendants: occasional single cells were seen to move out a head of expanding sheets of contiguous S-motile cells. S-motile strains have a strong tendency to form multicellular clumps in suspension and are difficult to grow fully dispersed in liquid culture. The same tendency was noted for SM by Burchard (1974), and is characteristic of *Myxococcus* strains freshly isolated from soil (Dworkin, 1966).

S-motile *cgl* mutants ($sglA^+$ cgl^-) were found to be stimulatable by contact with *agl* or different *cgl* mutant types as were their nonmotile *sglA1* cgl^- parents (Hodgkin and Kaiser, 1979). In this case stimulation led to the movement of single cells which was apparent in addition to the movement of groups of cells.

2. *Search for sgl Loci Different from sglA*

The sgl^+ agl^- and sgl^+ cgl^- S-motile strains described above were mutagenized and a new set of nonmotile mutants isolated to see if there were other loci with properties like *sglA*. Production of nonmotile mutants from a number of S-motile strains is summarized in Table 5. The same UV dose was used in these experiments as in the induction of mutants

Table 5. Induction of nonmotile mutants[a]

Strain	Genotype	No. of independent experiments	Total No. of colonies screened (approximate)	Total No. of nonmotile mutants isolated
a) AS- and A-motile strains				
DK100	+	2	5,000	0
DK101	*sglA1*	65	185,000	261
DK1258	*sglB3*	2	3,000	3
DK1253	*tgl-1*	2	6,000	5
b) S-motile strains				
DK1218	*cglB2*	11	32,000	39
DK1219	*cglC1*	14	45,000	75
DK1230	*cglE1*	3	5,000	2
DK1234	*cglF1*	1	3,000	8
DK1215	*aglA1*	1	1,000	2
DK1217	*aglB1*	1	7,000	9
DK1228	*aglC7*	1	3,000	7
DK1229	*aglD2*	1	9,000	4
DK1222	*aglE2*	1	4,000	4
DK1220	*aglF1*	1	2,000	0
DK1221	*aglG1*	1	2,000	1
DK1227	*aglH2*	1	3,000	2
DK1213	*aglN1*	1	3,000	5
DK1236	*aglR2*	1	2,000	4

[a] Mutations were induced by UV irradiation as described in Methods

Table 6. Multiple *sgl* loci

Recipient bacteria[a]	Transductional donor							
	None	DK100 (+)	DK101 (*sglA1*)	DK1266 (*sglE1*)	NM (*sglB9*)	DK1261 (*sglC1*)	DK1264 (*sglD1*)	DK1274 (*sglF1*)
DK1265 (*sglA2 cglB2*)	0		0		5			
DK1283 (*sglA3 cglF1*)	0	34	0	21				23
DK1243 (*sglA9 agl-12*)	0	12	1	7				8
DK1266 (*sglB1 cglB2*)	0		11		0			
DK1292 (*sglB3 aglR2*)	0	10	10	0				13
DK1261 (*sglC1 cglB2*)	0	2	2		2	0	9	
DK1264 (*sglD1 cglB2*)	0	6	7		4	15	0	
DK1244 (*sgl-44 aglA1*)	0	8	8	8				2
DK1245 (*sgl-45 aglG1*)	0	17	16	12				26
DK1248 (*sgl-48 aglB3*)	0	6	9	6				10
DK1260 (*sgl-60 aglR4*)	0	26	7	8				15

[a] 2.5×10^7 recipient bacteria were mixed with 10^8 to 2×10^8 phage Mx8 which had been grown on the donor strain, then treated as described in Methods for a standard motility transduction. Numbers in the table are the number of spots with S-motile flares among 55 total spots. (A-motile flares were present but were not counted.)

in DK101 and two other A-motile strains, DK1258 and DK1253, also shown in Table 5. In all cases, except DK100, nonmotile mutants were found at a frequency of about 10^{-3}.

A number of the nonmotile mutants derived from S-motile strains were examined further. When treated with transducing phage grown on DK100, all but one of them (65/66) gave rise to both A-motile and S-motile flares. The one exception gave only S-motile flares. It was found to carry an *mgl* mutation, i.e. is $cgl^- mgl^-$ and is described in more detail in Section 6 below.

These new nonmotile mutants are for the most part phenotypically similar. Most show less motility expressed as a more regular colony edge than the original set of nonmotile mutants derived from DK101 (Hodgkin and Kaiser, 1979), because the new *sgl* mutation are more extreme in effect than *sglA1*. Apparently the *sglA1* allele allows some degree of *sglA* expression, thus accounting for the movement observed at low temperature or on low nutrient of nonmotile mutants derived from DK101 (Hodgkin and Kaiser, 1977). Nonmotile mutants derived from temperature sensitive or unstable *agl* mutants exhibited the expected temperature sensitivity or instability. Two unusual types of nonmotile mutants have been observed. One has the stimulatable Tgl phenotype described below. A second produces colonies that appear bright and fuzzy under phase-contrast microscopic examination. These mutants tend to clump when grown in suspension, like their S-motile parents but unlike most nonmotile mutants.

How many different *sgl* loci are there? Table 6 reports a set of transductional crosses between mutants derived from several *agl* and *cgl* mutant strains, showing that transduction between strains differing at the *sglA*, *sglB*, *sglC*, *sglD*, and *sglF* loci give approximately as many flares as transduction from a DK100 donor into the same recipient. The *sgl* mutation in DK1265 is linked to *sglA1* and hence is assigned to the *sglA* locus but it is not the same as *sglA1* because DK1265 shows less irregularity of colony edge than the homologous *cglB2 sglA1* strain (DK321). Analogous crosses with a larger set of mutants derived from the *cglC1* strain suggest the existence of at least 7 loci, tentatively designated *sglA*, *sglB*, *sglC*, *sglD*, *sglE*, *sglF*, and *sglH* (data not presented). Strain DK1259 (*aglB1 sglG1*) contains a mutation at a putative eighth locus not detectably linked to any of these, but *sglG* mutations have not yet been isolated in *cglC* or *cglB* mutant backgrounds. Lesions at *sglA*, *sglB*, *sglC*, and *sglD* have been found among the nonmotile mutants isolated from both *cglB* and *cglC* mutants. Although more mutants and many more crosses are needed for definitive mapping, the data so far obtained do imply the existence of multiple *sgl* genes.

In the crosses used to distinguish *sgl* loci, A-motile transductants arose whenever donor and recipient differed at any *agl* or *cgl* locus. A number of these A-motile transductants were isolated and examined. They proved to be similar to each other and to DK101 in having mostly single cells at the periphery of their colonies.

3. An Hypothesis: Two Multi-Gene Systems Control Motility

Experiments in this and the preceding paper have demonstrated three distinct phenotypes: nonmotile,

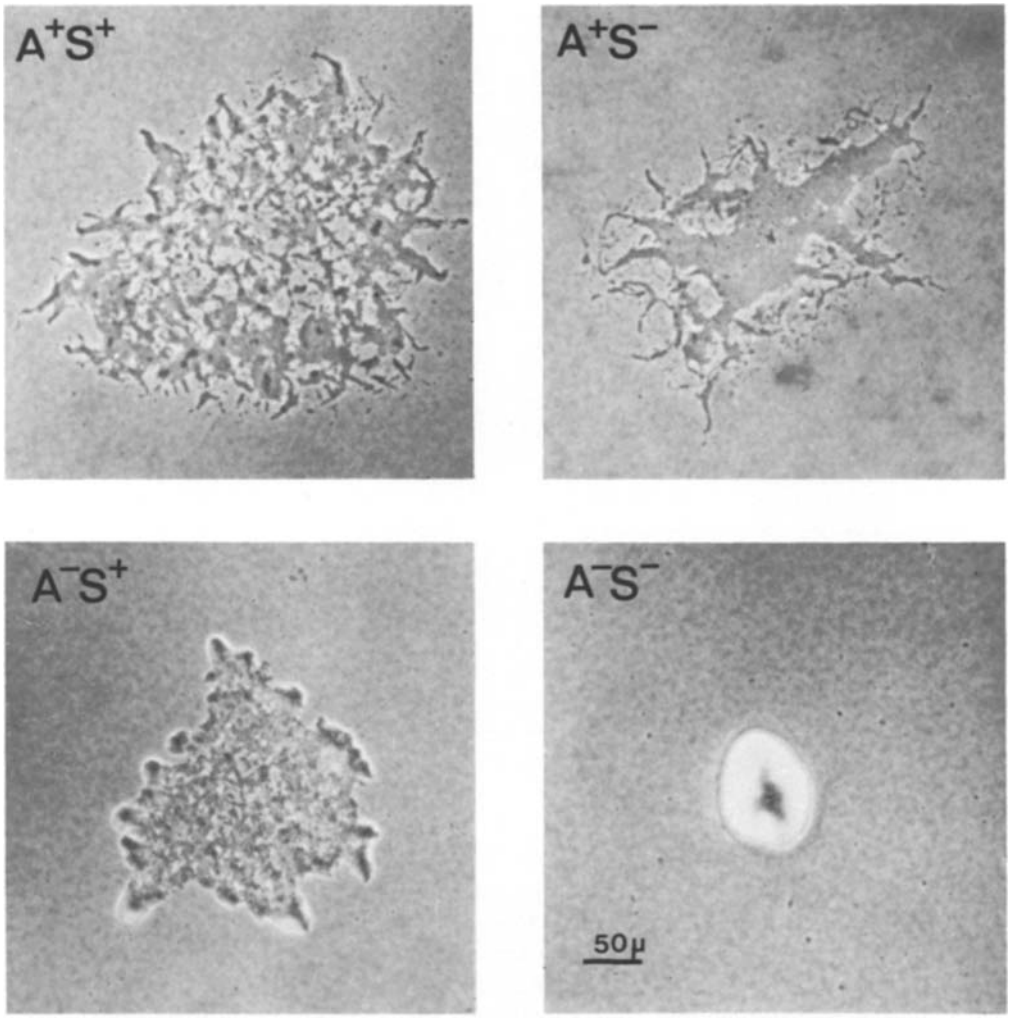

Fig. 2. Young colonies of three types of motility mutants and DK100 photographed after 40 h incubation at 32° C on CTT agar. The A^- mutation is *aglB1*, the S^- mutation is *sglG1*

A-motile (single cells moving), and S-motile (groups of cells moving). By means of mutation and transduction the three phenotypes have been found to be connected by two separate transition: A-motile⇄nonmotile⇄S-motile. Transitions between S-motile and nonmotile involve *sgl* loci and, as will be shown below, the *tgl* locus. No single-step transitions between A-motile and S-motile phenotypes have been observed.

These observations suggest that the genes controlling gliding motility in *M. xanthus* belong to two multi-gene systems. One, call it system A, controls the movement of single cells and includes all the *agl* and *cgl* loci. The other, call it system S, controls the movement of groups of cells and includes all the *sgl* loci and the *tgl* locus.

If a strain has wild type alleles at all of its system A loci, then that strain is said to be "A^+". Similarly "S^+" means wild type alleles at all system S loci. The major experimental basis for the two systems hypothesis is a direct correspondence between, on the one hand, an A^+ genotype and single cell movement, and, on the other hand, between an S^+ genotype and cell-group movement. This correspondence extends to wild type, A^+S^+, which has both single cells and groups of cells at the periphery of a colony. For clarity call this phenotype AS-motile. The four states of the two systems are illustrated by the photographs of young colonies presented in Fig. 2. The A^+S^- colony has many single cells at its periphery; but there are some thin finger-like groups of cells whose significance is considered in Discussion. The A^-S^+ colony has no single cells at the edge, only groups. The A^-S^- colony is a dense heap of cells without any fringe.

One prediction of the two systems hypothesis is that all double mutants which combine a mutation

Table 7. Phenotypes of known single and double mutants of system a and system S

		System S									
		S^+	*sglA*	*sglB*	*sglC*	*sglD*	*sglE*	*sglF*	*sglG*	*sglH*	*tgl*
System A	A^+	AS	A	A	A	A	A	A	A	A	A
	cglB	S	N	N	N	N	–	–	–	–	N
	cglC	S	N	N	N	N	N	N	–	N	N
	cglD	S	N	–	–	–	–	–	–	–	–
	cglE	S	N	–	–	–	–	–	–	–	N
	cglF	S	N	–	–	–	–	–	–	–	N
	aglA	S	N	–	–	–	–	–	–	–	N
	aglB	S	N	–	–	–	–	–	N	–	N
	aglC	S	N	–	–	–	–	–	–	–	N
	aglD	S	N	–	–	–	–	–	–	–	N
	aglE	S	N	N	–	–	–	–	–	–	N
	aglF	S	N	–	–	–	–	–	–	–	N
	aglG	S	N	–	–	–	–	–	–	–	N
	aglH	S	N	–	–	–	–	–	–	–	N
	aglJ	S	N	–	–	–	–	–	–	–	N
	aglK	S	N	–	–	–	–	–	–	–	N
	aglL	S	N	–	–	–	–	–	–	–	–
	aglM	S	N	–	–	–	–	–	–	–	–
	aglN	S	N	–	–	–	–	–	–	–	N
	aglP	S	N	–	–	–	–	–	–	–	N
	aglQ	S	N	–	–	–	–	–	–	–	?
	aglR	S	N	N	–	–	–	–	–	–	N

"AS" means AS-motile, both single cells and groups of cells move. "A" means A-motile, only single cells move. "S" means S-motile, only groups of cells move. "N" means nonmotile. ? see text for data on *aglQ tgl*

in one of the loci of system A with a mutation in one of the loci of system S should be nonmotile. Fifty different A^-S^- double mutants involving 8 different *sgl* loci, all 5 different *cgl* loci and all 16 known *agl* loci have been constructed as was described in the preceding sections and their motility properties are summarized in Table 7. All A^-S^- combinations were found to be nonmotile (with the possible exception of *aglQ tgl* which will be described below in Section 4).

Table 7 also shows that the 21 different A^-S^+ strains constructed exhibit the S-motile distribution of cells. They have only groups of cells at the edge of their colonies. One example, *aglB1*, was illustrated in Fig. 2. All 9 different A^+S^- strains exhibited the A-motile cell distribution, having mostly single cells at the edge of their colonies; one example, *sglG1*, was illustrated in Fig. 2.

4. *Stimulation in System S: Tgl Mutants*

If systems A and S are analogous, then there might be some S system mutants that would be stimulatable, just as the *cgl* mutants in system A are stimulatable. Detection of stimulation in the S system is facilitated by working in an A^- genetic background where A-motility will not obscure transient, stimulation-induced, S-motility. Stimulatable S^- mutants were sought among 75 nonmotile (A^-S^-) derivatives of 17 different *agl* strains, employed because they cannot be stimulated for A system motility (Hodgkin and Kaiser, 1977). Each A^-S^- strain was tested by mixing it with an *mgl* mutant (DK306), which had previously been shown to stimulate all *cgl* mutants (Hodgkin and Kaiser, 1977). Also some pairs of independent A^-S^- mutants were mixed with each other. Two of the 75 nonmotile mutants, DK1250 and DK1251, became transiently S-motile under these conditions (Fig. 3). The same two became transiently S-motile when mixed with other A^-S^- mutants but not when they were mixed with each other. Therefore, DK1250 and DK1251 belong to the same stimulation group. These mutants are designated *tgl*, to distinguish them from *sgl* mutants, which are not stimulatable.

In addition, 85 A^-S^- mutants derived from 4 different *cgl* strains were tested for capacity to be stimulated by mixing them with the corresponding *cgl sglA1* strain. Stimulation in system A does not occur because both members of each pair of strains carry the same defect in system A. One mutant, DK1252, derived from DK1234 (*cglF1*) became S-motile when mixed with DK370 (*cglF1 sglA1*) and hence was considered to carry a *tgl* mutation (*tgl-3*). It was possible

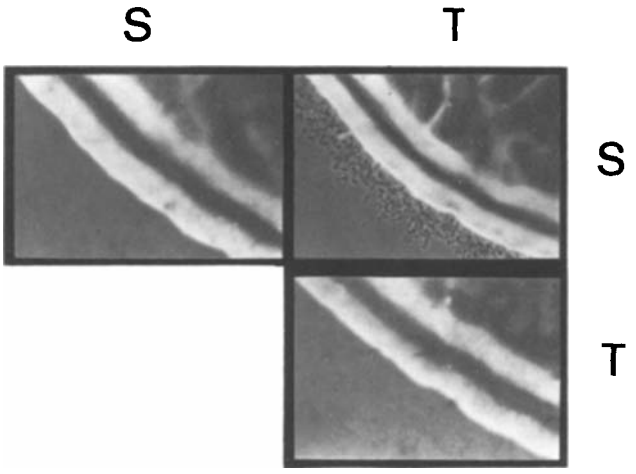

Fig. 3. Stimulation of *tgl*. A mixture of cultures of strains S and T, where S = DK1259 (*aglB1 sglG1*) and T = DK1250 (*aglB1 tgl-1*), is shown upper right. S alone is upper left and T alone is lower right. All were prepared according to the standard stimulation protocol. Photographs show a sector of the edge of the macrocolony formed by each culture. The edge runs from upper left to lower right. A fringe of S-motile cells is visible at the edge of the S plus T mixture but not in S or T alone

to look for system S stimulation of DK1252 by the *agl tgl* mutants described in the preceding paragraph, because the stimulation of system A in a *cglF1* strain by a $cglF^+$ strain is relatively weak (cf. Fig. 2 in Hodgkin and Kaiser, 1977). But no S-motility was observed when DK1252 (*tgl-3*) was mixed with either DK1250 (*tgl-1*) or DK1251 (*tgl-2*), although weak A-motility was observed as expected. Therefore, all three *tgl* mutants belong to one stimulation group.

Transductional crosses (Table 8) demonstrate that *tgl-1*, *tgl-2*, and *tgl-3* are closely linked to each other. Close linkage of the three *tgl* mutants was independently confirmed as a result of the discovery that *tgl* is frequently cotransduced with the locus responsible for rifampicin resistance as shown for *tgl-1* in Table 9. All three *tgl* isolates are linked to *rif* and, therefore, to each other. Six independent spontaneous Rif^R isolates were found to be linked to *tgl*, implying also that there is only one major locus for rifampicin resistance in *M. xanthus*.

The *tgl-rif* linkage facilitates strain construction by transfer of particular *tgl* mutations into any Rif^S strain. In practice, a spontaneous Rif^R mutant is selected from a *tgl* strain, and a transducing lysate of this double mutant is used to transduce rifampicin resistance into the recipient Rif^S. Approximately half of the resistant transductants are expected to carry the donor *tgl* locus. Twenty-one different S-motile strains, including both *cgl* and *agl* mutants were transduced to Rif^R from an *aglB1 tgl-1 rif-2* donor and data for four of them are presented in Table 9. In 20/21 crosses, some of the Rif^R transductants obtained were nonmotile. The finding that *agl tgl* and *cgl tgl* double mutants are in general nonmotile supports the hypothesis that motility is controlled by two independent systems of genes and that *tgl* belongs to one system and *agl* and *cgl* to the other.

The one case in which none of the Rif^R transductants were nonmotile involves DK1216 (*aglQ1*) and is included in Table 9. Although it is possible that *aglQ tgl* double mutants are S-motile contrary to expectation, it is also possible, since only 12 Rif^R transductants were obtained, that by chance none were *aglQ tgl*. Because of the absence of A-motile or AS-motile strains among the Rif^R transductants, the data show no evidence of linkage between *rif* and any of the *agl* or *cgl* loci tested in Table 8, but the data are too few to have revealed loose linkage.

The stimulation properties of some of the nonmotile Rif^R transductants were investigated to see whether they behaved as *agl tgl* or *cgl tgl* double mutants. They did; so for example the *cglC tgl* mutant was stimulated to AS-motility by an *mgl* mutant, which is expected to stimulate both *cgl* and *tgl* mutants. The *cglC tgl* double mutant was stimulated to A-motility by an *agl tgl* mutant, which is expected to be capable of stimulating only the A-system because both strains are tgl^-. Finally, the *cglC tgl* mu-

Table 8. Linkage of the *tgl* mutants

Recipient bacteria	Transductional donor					
	None	DK100 +	DK1250 (*aglB1 tgl-1*)	DK1251 (*aglJ1 tgl-2*)	DK1252 (*cglF1 tgl-3*)	NMM36 -1c
DK323 (*cglC1 sglA1*)	0	12	16	10	21	12
DK1250 (*aglB1 tgl-1*)	0	11	0	0	0	1
DK1251 (*aglJ1 tgl-2*)	0	4	0	0	0	0
DK1252 (*cglF1 tgl-3*)	0	33	2	0	0	1

Conditions and scores as in Table 6

Table 9. Cotransduction of *rif* and *tgl*

Recipient		Donor	Rif^R transductants[a]			% cotransduction
Strain	Phenotype		A	S	N	
DK1217 (*aglB1*)	S	*tgl$^+$ rif-1*	0	28	0	–
DK1217 (*aglB1*)	S	*aglB1 tgl-1 rif-2*	0	41	54	57
DK1250 (*aglB1 tgl-1*)	N	*tgl$^+$ rif-1*	0	18	16	53
DK1226 (*aglP1*)	S	*aglB1 tgl-1 rif-2*	0	41	3	7
DK1222 (*aglE2*)	S	*aglB1 tgl-1 rif-2*	0	41	12	23
DK1216 (*aglQ1*)	S	*aglB1 tgl-1 rif-2*	0	12	0	–
DK1230 (*cglE1*)	S	*aglB1 tgl-1 rif-2*	0	87	55	39

[a] "A" means A-motile or AS-motile; "S" means S-motile; "N" means nonmotile

tant was stimulated to S-motility by a *cglC sgl* mutant, which is expected to stimulate only the S-system because both strains are *cglC$^-$*.

A *tgl-1* strain (DK1253) was obtained as an A-motile (A^+S^-) transductant from the nonmotile recipient DK1250 (*aglB1 tgl-1*). As shown in Table 5, DK1253 gave rise to the expected frequency of nonmotile mutants. When these nonmotile mutants were tested in the stimulation assay, they behaved as expected for *agl tgl*, *cgl tgl*, or *mgl tgl* mutants.

Several experiments demonstrated that the stimulated movement observed in mixtures of *mgl$^-$ tgl$^+$* and *mgl$^+$ tgl$^-$* cells is due to the movement of the *mgl$^+$ tgl$^-$* cells only and is thus analogous to stimulation in system A in which only *cgl$^-$* cells move (Hodgkin and Kaiser, 1977). Cells of types *mgl$^-$ tgl$^+$* and *mgl$^+$ agl$^-$ tgl$^-$* carrying antibiotic resistance markers were mixed and placed on agar. Cells that had been stimulated to move (in all cases it was S-type motility) were picked up in fine glass capillary tubes, streaked out for single colonies, and tested for antibiotic resistance. When the *mgl* mutant strain was Rif^R and the *tgl$^-$* recipient was Rif^S all (291/291) of the colonies obtained from moving cells were Rif^S. In the inverse experiment, when the *tgl$^-$* strain was Rif^R, all (58/58) of the colonies derived from moving cells were Rif^R.

The stimulation of *tgl* mutants in system S more closely resembles the stimulation of *cglB* than *cglC* mutants in system A. First, stimulation was found to depend on contact with *tgl$^+$* cells for more than two hours. Second, formaldehyde-killed wild-type cells were unable to stimulate either *cglB$^-$* or *tgl$^-$* cells, though they could stimulate *cglC$^-$* cells (Hodgkin and Kaiser, 1977).

5. *The A and S Systems are Differentiated by the Capacity of Single Cells to Move*

To learn how the A- and S-motility patterns (illustrated in Fig. 1) arise, a series of strains representing the four states of the A and S systems were tested for the ability of single cells to move. Cultures were grown in liquid medium, diluted to very low density, plated on the surface of agar with carbon grains as position markers, and photographed over a 3 h interval. Single cells that were far enough away from all other cells to have never made contact with another were traced from one photographic frame to the next. Table 10 summarizes the results: single A^+S^+ and A^+S^- cells moved. In the A^+S^- category both a stimulatable (*tgl-1*) and a nonstimulatable (*sglG1*) strain were tested. Single cells of another non-stimulatable A^+S^- strain (*sglA1*) have previously been shown to move (Table 3, Hodgkin and Kaiser, 1979).

On the other hand, single A^-S^+ cells did not move (Table 10). This is true whether they were stimulatable A^- mutants like those carrying *cglB2*, *cglE1*, *cglF1*, *cglB7 cglD1*, or *cglC6 cglD1* or nonstimulatable A^- mutants like those carrying *aglH1*, *aglB1*, *aglE2*, or *aglR2*. The behavior of a strain which carries a temperature-sensitive mutation in an A system gene followed the pattern expected if A^+S^+ cells are, but A^-S^+ cells are not, motile when single. As shown to move (Table 3, Hodgkin and Kaiser, 1979). ature sensitive *aglJ1*, is motile as single cells if grown at 25° but nonmotile as single cells if grown at 33°.

Single A^-S^- cells are nonmotile (Table 10 and Table 3 of Hodgkin and Kaiser, 1979), also groups of A^-S^- cells must be nonmotile because the edges of A^-S^- colonies are sharp. Thus, there are two conditions for the motility of A^-S^+ cells: a complete set of system S genes and a group of contiguous cells. This suggests that the single cells found in the interior of an S-motile flare (Fig. 1) may have been dropped from a group of moving cells.

6. *The mgl Locus Belongs to Both Systems*

There is one function, and possibly only one function, shared by system A and system S. Ten nonmotile

Table 10. Movement of single cells

Genotype		Strain		No. cells traced[a]	No. cells moved	
					slightly[b]	≧1 length
A^+S^+		DK1050		497	154	162
A^+S^-	(*sglG1*)	DK1300		381	183	71
	(*tgl-1*)	DK1253		553	258	120
A^-S^+	(*aglB1*)	DK1217		357	11	0
	(*aglE2*)	DK1222		317	5	0
	(*aglN1*)	DK1213		328	6	0
	(*aglR2*)	DK1236		198	3	0
	(*cglB2*)	DK1218		338	3	0
	(*cglE1*)	DK1230		154	0	0
	(*cglF1*)	DK1234		264	6	0
	(*cglB7 cglD1*)	DK1237		381	3	0
	(*cglC6 cglD1*)	DK1238		391	1	0
A^-S^-	(*aglB1 sglG1*)	DK1259		230	0	0
	(*aglB1 tgl-1*)	DK1250		241	0	0
$A^{ts}S^+$	(*aglJ1*)	DK1212	25°	149	41	13
			33°	76	0	0

[a] Bacteria grown in liquid culture were diluted and deposited with carbon grains on $^1/_2$ CTT agar (or in a few cases CTT agar) in a closed chamber (either a small petri dish or a chamber on a microscope slide). The cultures were incubated at 26° and photographed at the beginning and end of a 3 h interval. Photographic negatives were projected and individual cells traced, using the carbon grains to superpose photographs of the same field taken at different times

[b] Cells are scored as having moved if one or both ends have displaced more than $^1/_3$ the cell length. "Slight" movement is taken as a displacement between $^1/_3$ and 1 cell length. Often this consisted of cell shortening or bending

mutants isolated from DK101 were found to be mutated at closely linked sites and were called *mgl* (Table 6, Hodgkin and Kaiser, 1979). These mutants differ from *cgl* or *agl* mutants in that they cannot by converted to the S-motile state: no S-motile transductant or revertant has been obtained from any *mgl* mutant derived from DK101. The *mgl* mutants of DK101 were observed to mutate or be transduced to the A-motile state as shown in Table 6 of the preceding paper, as were *cgl* and *agl* mutants. The absence of S-motile derivatives of *mgl* is not due to inefficient selection because reconstruction experiments, in which small numbers of S-motile cells were mixed with excess *mgl* cells, showed that the selection is as effective as with *agl* or *cgl* mutants (cf. Table 2).

A possible explanation for the behavior of the *mgl* mutants derived from DK101 is that mgl^+ (unlike cgl^+ or agl^+) is required for the functioning of both the A and the S systems. Thus, an *mgl* mutant of DK101, having the genotype *mgl sglA1*, could give A-motile mutants or transductants via the transition *mgl sglA1*→mgl^+ *sglA1*, but the other transition *mgl sglA1*→*mgl* $sglA^+$ would leave the strain nonmotile. This explanation would also account for the failure of *mgl sglA1* mutants to show the movement at low temperature or on low nutrient medium that is a characteristic of the phenotype of *sglA1* as described in Section 2 above.

If mgl^+ function is required for system S as well as system A then an *mgl* mutation should also render any A^-S^+ strain nonmotile. To examine this point, a large number of nonmotile double mutants that had been derived from *cglB2*, *cglC1*, *cglE1*, and *cglF1* strains were screened to see if among them were *cgl mgl* mutants, recognizable by the fact that, unlike their *cgl* parents, they would not be stimulatable. Fifty-eight nonmotile derivatives of *cglC1*, 17 of *cglB2*, and 8 of *cglF1* were screened. All these nonmotile strains could be stimulated to move as individual cells by the appropriate donor, and were, therefore, *cgl sgl* or *cgl tgl* double mutants. However, one of two nonmotile mutants derived from *cglE1* was unstimulatable, and was thus a candidate *cglE mgl* mutant. Transduction confirmed that this strain, DK1257, does indeed carry a mutation at the *mgl* locus (Table 11). As expected, DK1257 can be transduced or mutated to S-motility (*cgl mgl*→*cgl* mgl^+) but not to A-motility (*cgl mgl*→cgl^+ *mgl*) because a cgl^+ *mgl* mutant is nonmotile.

In addition a nonmotile mutant derived from DK1253 (*tgl-1*) was found to be unstimulatable, unlike other *tgl* mutants. This mutant (DK1256) also carries an *mgl* mutation, shown by the data of Table 11. As expected, transduction into DK1256 yields A-motile but no S-motile flares.

More than 400,000 colonies of *M. xanthus* have been screened in all the searches for nonmotile mutants described here and in the preceding paper and all those analyzed, more than 150 mutants, behave either as double mutants with a mutation in each system, or as single mutants at the *mgl* locus. These data support the idea that mgl^+ is essential for the proper function of both systems. In this light the paucity of nonmotile mutants from A^+S^+ strains compared to A^+S^- of A^-S^+ strains is reasonable. In an A^+S^- strain, a single mutation in any one of 22 or more loci results in nonmotility; in an A^-S^+ strain, a single mutation in any of 9 or more loci results in nonmotility; but in an A^+S^+ strain either a double mutation must occur or else a single mutation must arise within the confines of the *mgl* locus.

7. *Other Motility Mutants*

Motility mutants obtained by other investigators have been examined to see how they fit into the A and

Table 11. Linkage between *mgl* mutations
All crosses 5×10^7 bacteria and m.o.i. of 6. Scores are number of S-motile flares: number of A-motile flares.

Recipient bacteria	None	Transductional donor			
		DK100 A^+S^+	DK343 (*aglC7 sglA1*)	DK306 (*mgl-1 sglA1*)	DK1257 (*mgl-10 cglE1*)
DK343 (*aglC7 sglA1*)	0:0	27:44	0:0	0:25	43:42
DK306 (*mgl-1 sglA1*)	0:1	0:40	0:38	0:2	0:0
DK1256 (*mgl-11 tgl-1*)	0:0	0:16	–	0:0	0:0
DK1257 (*mgl-10 cglE1*)	0:0	18:0	17:0	0:0	0:0

S systems. Earlier crosses showed that Burchard's SM and NM contain a mutation at a known *agl* locus, *aglE3* (Table 7 in Hodgkin and Kaiser, 1979). Furthermore, NM contains an additional mutation at *sglB* (see Table 4). We have been able to generate A-motile but not S-motile transductants from NM, suggesting that it may contain two unlinked mutations in system S. Thus SM is of the type A^- and NM of the type A^-S^-. This is consistent with the origin of NM; Burchard (1974) obtained it as a mutant of SM.

Four mutants described by MacRae and McCurdy (1976) are genetically more complex. When treated with a transducing stock prepared on DK100, mutant NMM36-1c gave S-motile but not A-motile or AS-motile transductants, while mutants -3d, -5L, and -6J gave no motile transductants of any sort. All four strains are sensitive to phage Mx8; therefore, the failure to obtain motile transductants suggests that these strains may be multiple mutants. Data on the frequency with which mutants of this type arise were not given in the published description, but the procedure used for the induction and selection of these mutants might have generated multiple mutations or deletions. Transducing phage stocks were grown on the four strains and these stocks were tested on some of the standard mutants in our collection. The transduction results, some of which were presented in Table 4, indicate that NMM36-3d carries mutations at *sglA* and probably *aglJ*; NMM36-5L at *cglB* and *tgl*; NMM36-6J at *sglA* and at a locus linked to *aglC*; NMM36-1c at *aglJ* and *tgl*. All four are mgl^+. However, these mutations only partly explain the phenotypes of the NMM36 series of mutants. For example, NMM36-5L probably carries a second, nonstimulatable, A^- mutation and a second, nonstimulatable, S^- mutation because it cannot be stimulated to move. NMM36-5L cannot stimulate either $cglB^-$ or tgl^- cells, as expected for a *cglB tgl* multiple mutant. NMM36-1c can be stimulated to S-motility by all S^- mutants tested except tgl^-, as expected for a *tgl* mutant. Thus, all four mutants are A^-S^-. Possibly NMM36-3d, -5L and -6J carry two mutations in different genes of system A and two in different genes of system S and -1c carries two mutations in system A and one in S. Alternatively, they may be double mutants carrying deletions that prevent recombination at the relevant loci. In either case, all four appear to carry mutations both in system A and in system S, and their nonmotility can be accounted for within the framework of these two systems. Like DK101, NMM36-3d and strain YNS probably carry a mutation at *sglA* (Table 4).

8. *Effects on Fruiting*

The existence of two systems of motility control raises the question—why two? One possibility is that the A and S systems play different roles in the major coordinated movements, swarming and fruiting, that *M. xanthus* executes. To pursue this question the effects of A^- and S^- mutations on fruiting have been examined. In one experiment 40 independent A^-S^- double mutants, having genotypes *cgl sgl*, *agl sgl*, and *mgl sgl* were tested. None were able to fruit. Although most did form myxospores, they arose in disorganized lawns rather than in discrete fruiting bodies. One or two of the more unstable mutants did show some aggregation.

It is not surprising that nonmotile mutants fail to fruit because fruiting obviously requires movement. A more critical question is whether either A^-S^+ or A^+S^- strains can fruit. A number of such strains were tested under standard fruiting conditions and the results are presented in Table 12 and Fig. 4. The

Table 12. Fruiting response of motility mutants

Genotype	Number of strains tested	Fruiting*		
		Good	Poor	None
A^+S^+	1	1	0	0
A^-S^+	39	35	2	2
A^+S^-	36	12	6	18

Fruiting conditions are described in the legend to Fig. 4. *Good fruiting means large fruits as in Fig. 4 (4). Poor fruiting means small or poorly formed fruits as in Fig. 4 (m). No fruiting as Fig. 4 (s)

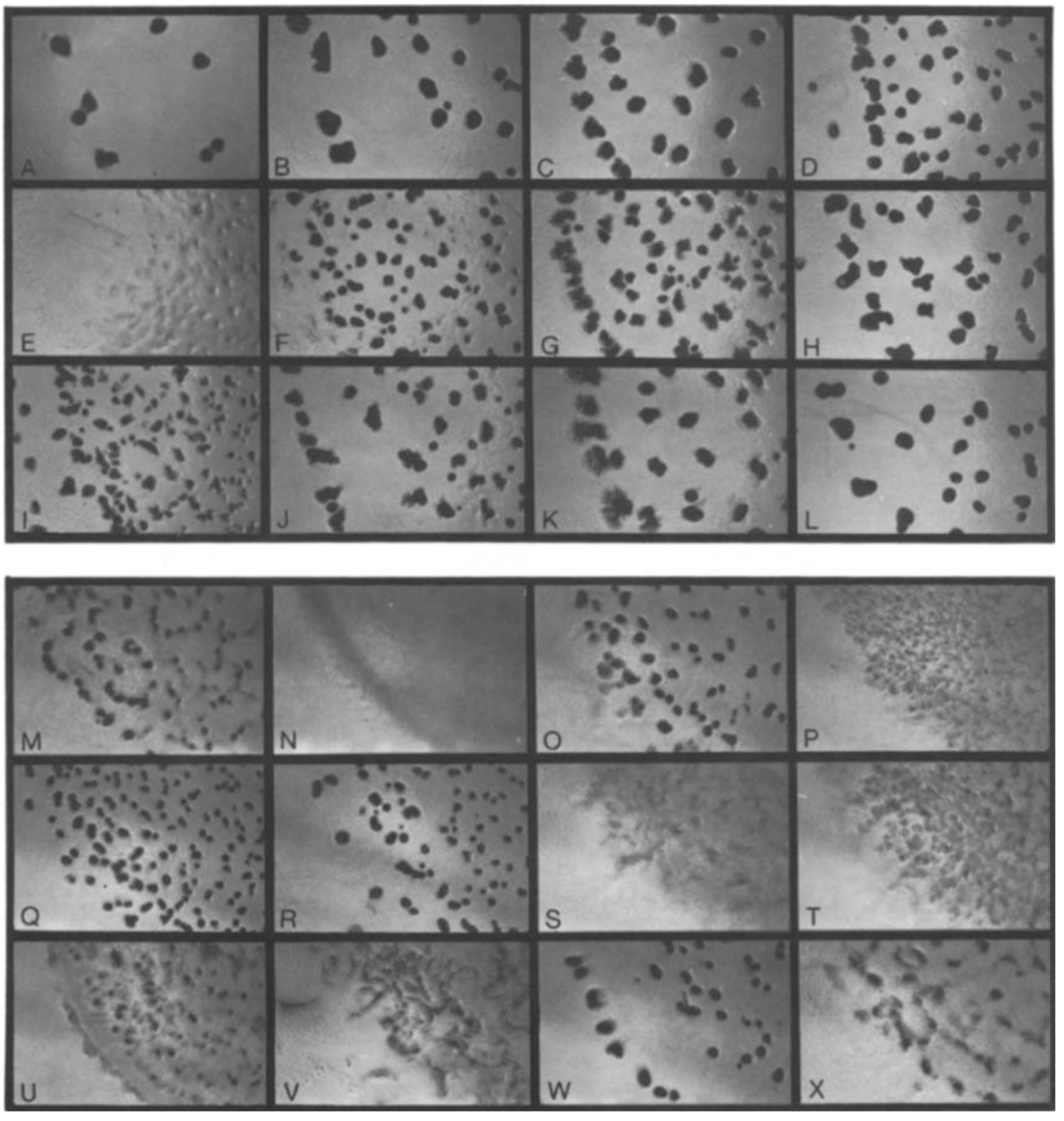

Fig. 4. Fruiting behavior of motility mutant strains compared with that of the wild type. All strains were grown in CTT broth to midlog phase and concentrated by centrifugation to 10^9 cells/ml. Drops of 5 µl were placed on PM plates and incubated $3^1/_2$ days at 32° C, then photographed. (*a*) A^+S^+ (DK100); *b* through *l* [letter] are A^-S^+ strains carrying mutations *aglD2* (*b*), *aglC7* (*c*), *aglC1* (*d*), *aglH2* (*e*), *aglJ1* (*f*), *aglQ1* (*g*), *aglN1* (*h*), *cglF1* (*i*), *cglE1* (*j*), *cglC1* (*k*), *cglB2* (*l*); *m* through *x* are 12 different A^+S^- strains carrying mutations: *sglF74* (*m*), *sglB75* (*n*), *sglA76* (*o*), *sgl-77* (*p*), *sglD78* (*q*), *sgl-79* (*r*), *sglB80* (*s*), *sgl-81* (*t*), *sglC1* (*u*), *sgl-94* (*v*), *sglB67* (*w*), *sgl-68* (*x*)

39 A^-S^+ strains tested were independent isolates, with known *agl* or *cgl* defects representing all 21 *cgl* and *agl* loci. Almost all 39 fruited well, although the fruiting bodies were usually smaller and more closely spaced than wild-type fruiting bodies as shown by the photographs exhibited in the upper half of Fig. 4. Two mutants showed little or no aggregation, DK1227 (*aglH2*, panel e) and DK1232 (*aglM1*, not shown in Fig. 4). It was noted above in section 1 that mutants at *aglH* and at *aglM* have less capacity to spread than SM, as if these mutations have a slight effect on system S in addition to a strong effect on system A.

In contrast to the ability of A^-S^+ mutants to fruit well, two-thirds of the A^+S^- mutants tested fruited poorly or not at all (Table 12 and Fig. 4, lower half).

These observations should be regarded as tentative for several reasons. The criteria for good fruiting are qualitative since they depend on an assessment of the morphology of fruiting bodies. Also we have not yet investigated the effect of varying conditions known

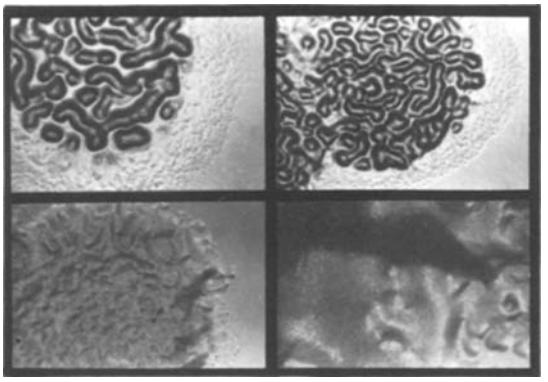

Fig. 5. Aggregation in colonies of A^-S^+ strains. (Upper left) DK1218, *cglB2*; (upper right) DK1217, *aglB1*; (lower left) DK1219, *cglC1*; (lower right) DK100, A^+S^+. The edge of the A^+S^+ colony is visible in the lower right corner. Colonies were grown 6 days at 27° on CTT agar containing 0.75% rather than 1% casitone and photographed at 10X

to affect fruiting, such as time of incubation, cell concentration, and nutritional levels on the fruiting of the various mutants. Nor have we determined how much motility is exhibited by the mutants under the starvation conditions used to test fruiting. Moreover, it was possible to choose A^-S^+ mutants to represent all the known loci in system A, and that system appears to be close to genetic saturation. But genetic characterization of system S is less advanced; most probably only some of the loci have been identified. For all these reasons, the fraction of A^+S^- mutants that failed to aggregate (18/36) or aggregated poorly (6/36) should be considered a rough approximation to the fraction of S system loci whose proper function is required for aggregation. Nevertheless, many S^- mutations do seriously impair the capacity to fruit, whereas few A^- mutations seem to.

Another piece of evidence suggestive of different roles for systems A and S is provided by behavior under conditions of reduced nutrition. On agar containing 0.75% Casitone A^-S^+ strains form aggregates that resemble early stages in fruiting but that lack myxospores (Fig. 5). Similar aggregates were not formed by A^+S^- strains examined under the same conditions. The clumping of S^+ strains grown in suspension, noted in section 1, may be a related phenomenon.

Discussion

Evidence is presented here for a group of genes, called motility system S, that controls the gliding of groups of cells. System S encompasses at least 9 genes: 8 *sgl* loci, *A*, *B*, *C*, *D*, *E*, *F*, *G*, *H*, and one *tgl* locus. Mutants at the *tgl* locus are stimulatable by contact with other mutants to produce cell movement. Motility in *M. xanthus* combines the function of system S with that of system A which controls the gliding of single cells and includes 5 *cgl* loci and 16 *agl* loci (Hodgkin and Kaiser, 1979). One locus, *mgl*, is required for the function of both systems. These relationships are summarized in Fig. 6.

There are several reasons for dividing the loci that affect motility into two separate gene systems. Single mutants in any of the loci (except *mgl*) are still motile, although their pattern of movement differs from that of wild type. Double mutants with both mutations in genes that belong to the same system, for example, *cglC6 cglD1* and *cglB7 cglD1*, are motile. But all double mutants that combine a mutation in any locus of system A with a mutation in any locus of system S are nonmotile. Fifty different locus combinations have been made and are all nonmotile, with one possible exception (Table 7).

Another reason for dividing the known motility loci into A and S systems is that single A^+S^- cells can move, but single A^-S^+ cells cannot. This point is demonstrated by the data of Table 10 and by the distribution of cells at the edge of colonies of various mutants, because at the edge of a colony movement has a greater effect than growth. Cells at the edge of an A^+S^- colony or flare are mostly isolated from each other, whereas cells of an A^-S^+ colony or flare are all in raft-like groups (Fig. 1, Fig. 2). These are the characteristic A-motile (single cells moving) and S-motile (groups of cells moving) phenotypes. Wild type *M. xanthus* (A^+S^+) combines the two patterns: it has both many single cells and many groups of cells at edges (AS-motile).

There are some single cells in the interior of an A^-S^+ flare (Fig. 1). They may have been dropped from groups of moving cells. There are some finger-like groups of cells at the edge of an A^+S^- flare or colony (Figs. 1, 2). Consequently, one should not lose sight of the possibility that A^+S^- cells are capable of some group movement. However, the presence of cell groups is not incompatible with pure single cell movement. For example, such groups might arise division of stationary cells, by transient association of independently moving cells, or as a consequence of the propensity of myxobacterial cells to follow slime trails laid down by preceding cells (Kühlwein and Reichenbach, 1968).

Despite their differences the A and S systems have major similarities. The two systems seem to have comparable numbers of genes because A^- and S^- mutants arise at roughly the same frequency (c.f. Table 5). Certain mutants in both systems, the *cgl* mutants in system A and the *tgl* mutants in system S, are conditional defectives in the sense that they can be stimulated to move, transiently, by contact with other mutants. In both systems there is a one to one correspon-

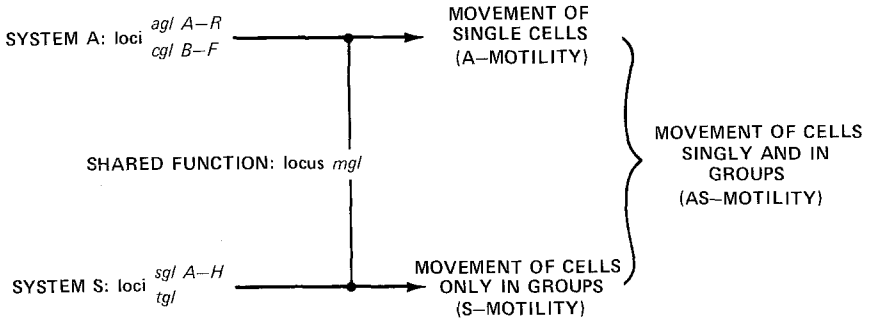

Fig. 6. Two gene systems, A and S, govern motility in *M. xanthus*

dence between stimulation type and mutant locus. For example, four stimulatable (*tgl*) mutants in system S have been isolated. None of these mutants stimulate each other, implying that they are defective in the same, or very closely related, function(s). All four mutations are linked by transduction tests, defining a single *tgl* locus. Similarly in system A all *cglB* mutants belong to the same stimulation group and are closely linked; likewise for *cglC*, *cglD*, and *cglE*. Only one *cglF* mutant has been isolated so far.

The finding of two independent systems of motility control is striking and was unexpected as it is not the case in *E. coli* or *Salmonella typhimurium* (Iino, 1977; Parkinson, 1977; Silverman and Simon, 1977). One possible reason for the existence of two parallel systems is suggested by the fruiting behavior of A and S mutants: in general, A^-S^+ mutants fruit well, while A^+S^- mutants fruit poorly or not at all. Cultures of A^-S^+ strains are more cohesive and have a greater tendency to clump than A^+S^- strains. Based on these properties we suggest that one role of system S is to facilitate fruiting by increasing the aggregation of cells.

An alternative explanation for the existence of two systems of motility control is that they represent two different "machines" for gliding rather than two parallel controls on a single mechanism. If one mechanism were unable to function under certain conditions, at high cell density for example, then there would be selection for a second mechanism that could so function. On this hypothesis one would expect that other gliding prokaryotes that form swarms, including those from non-fruiting orders (e.g., *Vitreoscilla*, *Cytophaga*), might also have some kind of dual system. In fact, gliding bacteria other than myxobacteria do frequently show cooperative movement (Henrichsen, 1972). Species in the genus *Cytophaga* seem to prefer to move in groups (Stanier, 1940) and conditional as well as nonconditional mutants have been identified (Glaser and Pate, 1973).

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