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23. *Sci. News* **98**, 364 (1970); (*1*, pp. 65–69).
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25. As a participant in the 1970 Calico Conference, I submitted an earlier version of this article to the San Bernardino County Museum for inclusion in the conference report. Because of its length it was rejected, and, at L. S. B. Leakey's request, publication was withheld until after the official report was in print. I wish to express my appreciation to the National Geographic Society, which sponsored my visits to the Calico site in 1963, 1965, 1966, and 1967; to the Institute for the Study of Earth and Man of Southern Methodist University for making it possible for me to attend the 1970 Calico Conference; and to the San Bernardino County Museum and the Calico staff and crew for their courtesies and hospitality during my visits to both the Museum and the site. Reading of the manuscript and constructive commentary by C. C. Albritton, E. W. Haury, and J. L. Shiner is also appreciated.

Control Circuits for Determination and Transdetermination

Bistable control circuits like those in bacteriophage lambda may function in *Drosophila* development.

Stuart A. Kauffman

In this article I propose a systematic theory for major aspects of imaginal disk determination and transdetermination in *Drosophila melanogaster*. Over the past decade experiments on these processes have revealed striking phenomena. *Drosophila* is a holometabolous insect with four major developmental stages. Embryonic development persists for about 22 hours after fertilization, then the larva hatches, grows for about 4 days, forms a pupa, and in 4 days undergoes metamorphosis to an adult. During metamorphosis many larval tissues break down and most portions of the adult's surface are formed from small structures in the larva called imaginal disks. Imaginal disk determination is the paradigm of normally irreversible commitment. Each disk is a small nest of cells set aside early in embryogenesis which is determined to develop during metamorphosis into a particular adult structure: wing, leg, or antenna, for example. At the time of determination, each disk consists of about 10 to 40 cells (*1*). During the ensuing three larval instars, the disks proliferate and by the third instar each consists of several thousand cells. Disk cells remain undifferentiated and never form a functional part of the larva. Despite their proliferation, each disk maintains its determined state and differentiates appropriately during metamorphosis.

Several years ago Hadorn (*2*) tested the capacity of disk tissue to maintain its determined state for very long periods of time by excising genital disks from third instar larvae and growing them in the abdomen of adult flies where they proliferate but do not differentiate. Tissue lines were carried by serial transfers in adults, and the maintenance of determination tested by implanting tissue fragments into larvae which then underwent metamorphosis. Hadorn found that even after several years, tissue initially derived from genital disks still metamorphosed to adult genital tissue. This important result demands that whatever the molecular carriers of determination may be, the determined state is a persistently regenerated, self-maintained state.

Hadorn also discovered that tissue lines initially derived from genital disks sometimes transdetermined and gave rise, when tested by metamorphosis, to adult tissue normally derived from another disk. Hadorn and his co-workers (*3–10*) found that each type of transdetermination, from genital to antennal structures, or from leg to wing structures, for example, occurs with a characteristic probability per transfer generation from adult to adult, and that the new state of determination is clonally heritable in the transdetermined tissue line. Evidence that transdetermination is not due to somatic mutation is provided by the discovery that transdetermination occurs in groups of contiguous cells not clonally related (*8*), and is too frequent to be due to mutation.

There are a number of striking features of the transdetermination flow pathway (Fig. 1). The most important is that there are specific sequences of transdeterminations. Genital disks transdetermine into antennal or leg structures; antennal or leg disks in turn transdetermine into wing structures; wing disks then transdetermine into mesothorax structures. Genital disks do not transdetermine directly into wing or mesothorax structures. The flow pathway gives evidence of a distance measure (that is, a measure of the degree of difference) between the states of determination in various disks. Haltere disks transdetermine directly to wing, but not to leg or genitalia; leg disk transdetermines directly to wing, but not to haltere. In some sense haltere is closer to wing than to leg

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and genitalia; both leg and haltere are close to wing but not to each other. A fundamental property of transdetermination is that it is asymmetric, for several of the transdeterminations are reversible, but with considerably different frequencies.

The conclusion that the determined state must be a self-maintained state provides a natural explanation for the characteristics of the transdetermination flow pathways noted above. Whatever the molecular circuits that maintain determination may be, whether they function by transcriptional control, translational control, DNA packing, or unforeseen mechanisms, almost any plausible control circuit has the property of possessing sharply distinct alternate stationary states, rather than a continuous range of stationary states as exemplified by a frictionless pendulum that can oscillate at any chosen amplitude. These properties are exhibited in bacteriophage lambda by a bistable control circuit that is dominated by two control genes, C_1 and *tof*, which mutually repress one another through intervening processes (*11, 12*). This circuit has two distinct self-maintained stationary states: C_1 on, *tof* off; C_1 off, *tof* on. The former, the immunity (im+) state, corresponds to the lysogenic state, the latter im$^-$ state corresponds to the lytic sequence and only exists as a stationary state in defective lysogens unable to lyse. Either regulatory state may persist in infected *Escherichia coli* over indefinite generations, and either may change spontaneously at a low frequency to the other regulatory state (*12*), a behavior reminiscent of transdetermination.

If the control circuit or circuits underlying determination in each disk each have sharply distinct stationary states, then the natural description of the determined state in each disk consists in specifying which alternate state of each circuit occurs in that disk. If more than one circuit is involved, then the state of determination in each disk is characterized by the combination of states chosen by the different circuits. In turn, this implies a natural symmetric distance measure between the states of determination in various disks consisting of the number of circuits that must change state in order to change the determined state of one disk into the detemined state of another disk. This leads to the expectation that there will be sequences of transdeterminations, for if transdetermination from disk A to B involves changing the states of some circuits, while that from A to C involves changing not only those same circuits, but additional circuits, then B is between A and C. Transdetermination should occur in the sequence A to B to C. Asymmetry in reversible transdetermination frequencies is then a reflection of inequality in the relative stabilities of the alternate stationary states of each circuit and asymmetric transition frequencies between them.

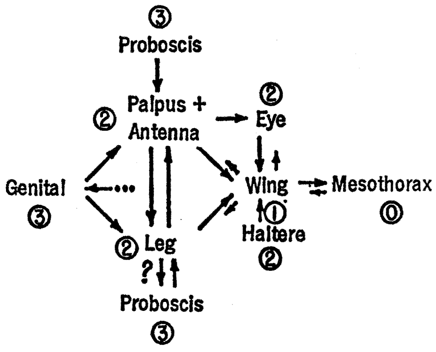

Fig. 1. Transdeterminations among the imaginal disks of *Drosophila melanogaster*. Lengths of arrows represent relative frequencies of transdetermination. Encircled numbers indicate the minimum numbers of transdetermination steps needed before the disk can reach the determined state of mesothorax. Transdetermination from leg to proboscis is uncertain. Dotted arrow to genital signifies that tissue from which the transdetermination occurred is not known. The transdetermination from proboscis to leg (*6*), between antenna and eye (*7, 8, 14*), and from mesothorax to wing (*9*), have been added. [After Gehring (*10*)]

This general characterization of the phenomena of determination and transdetermination emphasizes a number of questions: How many control circuits underlie disk determination? How many alternative stationary states does each circuit possess? What are their relative stabilities? What state of each circuit occurs in each disk's determined state? While our ultimate aim is to discover the molecular character of these circuits, that goal is distant. Discovery of such "macroscopic" properties of these circuits appears experimentally feasible without our knowing in detail the microscopic construction, and should aid in the elucidation of the molecular character. In the remainder of this article I provide evidence that determination is underlain by a number of circuits each with only two alternative stationary states, one considerably more stable than the other, that there are at least four such circuits, and that one can make tentative state assignments for all the circuits in each disk.

(A)	1	2
1	.5	.5
2	.3	.7

(B)	1	2	3
1	.5	.01	.49
2	.3	.7	0
3	—	—	—

Fig. 2. Markov transition matrices for a two state (A), and a three state (B), system. Each row shows the probability that the corresponding state remains unchanged, or changes to each of the other states during the next time interval. State 1 is less stable—more likely to change—than state 2 in both (A) and (B). Therefore the probability that state 1 will change to state 2 is greater than the reverse in (A). This is not true in (B), where the more stable state, 2, is more likely to change to the less stable state, 1, than the reverse.

Bistable Memory Circuits

The transdetermination flow pathways in Fig. 1 exhibit an important feature not yet discussed. Each disk is labeled by the minimum number of transdetermination steps that it must undergo to reach mesothorax: mesothorax, 0; wing, 1; haltere, eye, antenna, and leg, 2; proboscis and genital, 3. With the possible exception of the reversible transdeterminations between leg and proboscis (*6*), every transdetermination from a structure located further from the mesothorax to a structure one step closer, is more probable than the reverse. There is an unmistakable global orientation to the entire flow pathway.

I suggest that the most natural hypothesis to account for this global orientation requires that the processes of disk determination and transdetermination be underlain by control circuits with at most two stationary states, one more stable than the other. These requirements follow from the fact that with only two stationary states, the transition probability is always greater from the less stable state (that is, the state more likely to change) to the more stable stationary state, than the reverse. This guarantee is lost as soon as the circuit has three or more stationary states, for the matrix of transition probabilities has two or more degrees of freedom in each row (Fig. 2). Therefore, the transition from a less stable to a particular more stable stationary state may be less probable than the reverse. In particular, were disk determination due to eight stationary

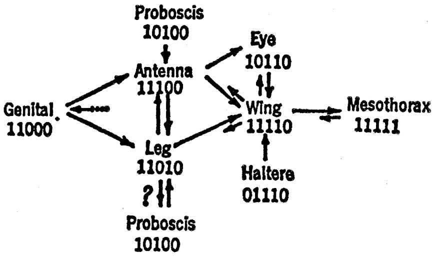

Fig. 3. Simplest model to account for oriented transdeterminations toward mesothorax in terms of bistable circuits. State "11111" refers to five distinct circuits, each in its more stable state.

states of a circuit, one for each type of disk, then even were there a gradient of stabilities with the mesothorax being the most stable, there would be no reason to predict the oriented transdeterminations toward mesothorax from each disk. For example, were the wing the next most stable disk, there would be no reason to predict that transdetermination from wing to mesothorax is more frequent than from mesothorax to wing.

By contrast, if disk determination were underlain by circuits each with only two stationary states, one more stable than the other, then any transdetermination in which one or more circuits changed from its less to its more stable state would be more probable than the reverse. To obtain the global oriented flow, for a first, simple, model, suppose each single step nearer mesothorax changes a single circuit from its less to its more stable stationary state, and each disk is characterized by a unique combination of circuit states. Five circuits are required because four structures are one transdetermination step from wing and wing one from mesothorax. If "1" represents the more stable stationary state of an entire circuit, such as (*tof* on, C_1 off), and "0" the less stable stationary state, one obtains the model in Fig. 3. There are no free choices except for the order of listing the five circuits, and for proboscis.

By construction, the model creates the oriented flow toward mesothorax. Although constructed merely to fit the global orientation, the model also fits a number of other quantitative relations between transdetermination probabilities (see Fig. 4). For example, the model predicts that transdetermination from leg to wing should be more probable than transdetermination from leg to antenna, because leg to wing changes the third circuit from 0 to 1 as does the transition from leg to antenna, but the latter transition also changes the fourth circuit from 1 to 0. This prediction appears to be true (*4*), because the probability of transdetermination from leg to wing was .64 while that from leg to antenna was .033 (Table 1 and Fig. 4).

Of the 33 quantitative relationships between transdetermination frequencies derived from the model (Table 1 and Fig. 4) data exist for 27 and 19 appear to be true. However, I should stress that it is generally difficult to obtain reliable and comparable quantitative measures of transdetermination frequencies. Those frequencies reflect such factors as the genotypes of host and donor, and are positively correlated with the extent to which the initial donor disk is disaggregated and proliferates in culture (*4*). The transdetermination frequencies in Fig. 4 for leg and wing (*4, 13*), antenna (*8, 14*), and haltere disks (*3*), are from experiments on the gentoype *e, mwh*, in which the initial disks were disaggregated in the same manner. The disaggregated disks were cultured for short periods varying from 16 to 42 days before being implanted in metamorphosing larvae. Transdetermination from genital disks was tested under various conditions (*2, 5, 13*); the data were then averaged and are therefore not directly comparable to the others (*15*).

Table 1. Predictions generated from the model in Fig. 3 (T, true; F, false). A→W > A→L means "transdetermination from antenna to wing should be more probable than transdetermination from antenna to leg." Predictions that two transdetermination frequencies should be equal are difficult to assess with variable data, and are not listed. Of ten that might be made, four appear to be true, four uncertain, and two (A→W = P→E, G→L = P→E) false. S, status.

Predictions	S	Predictions	S
L→W > L→A	T	H→W > H→A	T
W→A > L→A	T	P→A > P→L	?
A→W > A→L	T	H→W > H→E	T
W→L > A→L	F	W→E > H→E	T
L→W > L→E	T	E→W > E→H	T
W→E > L→E	T	W→H > E→H	?
E→W > E→L	T	A→W > A→E	T
W→L > E→L	T	W→E > A→E	F
L→W > L→H	T	E→W > E→A	?
W→H > L→H	?	W→A > E→A	?
H→W > H→L	T	If [G→A > G→L], then [L→W > A→W]	T
W→L > H→L	T		
A→W > A→H	T	A→G > A→L	F
W→H > A→H	?	L→G > L→A	F
P→A > P→G	T	P→G > P→L	F
G→A > G→P	T	P→E > P→L	F
P→G > P→L	F	P→W > P→L	F

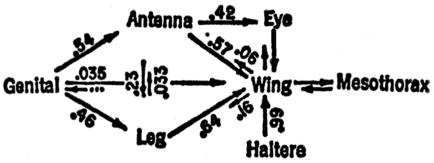

Fig. 4. Observed transdetermination frequencies. Data are from (*4, 13*) for leg and wing disks; (*8, 14*) for antenna disks; (*2, 5, 13*) for genital disks; and (*3*) for haltere disks. Reliable and comparable quantitative measures of transdetermination frequencies are difficult to obtain.

The predictions listed in Table 1 are independent of the data used to construct the model. For the model I used the minimum number of transdetermination steps needed to reach mesothorax from each disk, taking into consideration the observation that each transdetermination one step toward mesothorax is more probable than its reverse. Neither the knowledge that leg, antenna, eye, and haltere are one step from wing and two from mesothorax, nor the observation that transdeterminations from them to wing are more frequent than from wing to them, provides any information about the frequencies of transdeterminations between antenna, leg, eye, and haltere. Therefore, predictions that transdetermination from leg to wing is more frequent than leg to antenna or leg to eye are tests of the model. Nevertheless, when only the data on transdetermination frequencies are used to construct and test a model, the model remains unavoidably a partial redescription of those data. An independent approach is needed.

The Distance between Determined States

In my analysis of disk determination in terms of circuits with two stationary states I interpret the distance between the determined state of two disks as the number of circuits that must change state in order to change the determined state of one disk into the determined state of the other. In the model, in Fig. 3, the haltere disk is a distance of one from the wing disk, and three from the genital disk. Experimental data are required that are totally independent of transdetermination probabilities, that support the hypothesis that disk determination is underlain by circuits with at most two stationary states, and that give means independent of transdetermination frequencies to measure distances between the determined states of the disks in terms of the states of their circuits.

It should be possible to discover if any memory circuit underlying disk determination possesses two stationary states. If a circuit has *K* stationary states, that circuit partitions the set of disks into at most *K* disjoint subsets, those disks in which the circuit is in state 1, state 2 . . . , state *K*. If the memory circuits underlying disk determination have two stationary states, then each circuit partitions all the disks into two disjoint, complementary subsets, those disks in which the circuit is in state 1, and the rest in which it is in state 0. Mutations should occur that make state 1 alone, or state 0 alone, abnormal. Therefore, if each memory circuit has two stationary states, it should be possible to find pairs of mutants in which one member of each pair affects one subset of disks, while the other member of each pair affects all the remaining disks. For this argument it is not necessary to demand that the mutations affect state 1 or state 0 of the control circuit itself. It is sufficient if the mutations affect processes uniquely associated with state 1 alone, or state 0 alone. Any such process would serve as a sign of its associated circuit state, and mutations affecting these processes should affect complementary subsets of disks.

The predicted existence of pairs of mutants acting on complementary subsets of disks differs markedly from the prediction that comes from supposing a particular gene product to be required in more than one disk, which predicts only that a single mutation will affect more than one disk, but gives no reason to expect another mutation to affect all and only the remaining disks.

Shearn *et al.* (*16*) have collected 34 lethal mutants of the late larval stage of *Drosophila* in which some disks are normal, while others are abnormal in developmental potential, or are abnormal in both morphology and developmental potential. The 34 mutants affecting subsets of five disks fall into 13 classes (*17*) (Table 2). Of these 13 classes, eight from four complementary pairs, each of which jointly exhausts the set of disks examined. For example, one class affects only haltere and wing disks, another affects only antenna, eye, and legs. The two jointly exhaust the five disks routinely examined.

The natures of the mutant phenotypes differ. Of the eight mutant classes which form complementary pairs, four have normal disk morphology, but abnormal development, two are abnormal in both respects, one causes absence of two disks, and one causes a homeotic alteration of haltere disk to give wing tissue and genital disk to give leg and antenna tissue.

Table 2. Thirteen classes of mutants [Shearn *et al.* (*16*) and Shearn (*17*)] each affecting the development of a subset of the eye (E), antenna (A), haltere (H), wing (W), and leg disks (L). The first four pairs affect complementary subsets of disks. The last column gives the number of mutants in phenotype classes: *A*, disk absent; *B*, disk present but morphologically and developmentally abnormal; *C*, disks morphologically normal but developmentally abnormal; *D*, homeotic effect.

Class	E	A	H	W	L	Type
1	X	X				2*C*
2			X	X	X	1*C*
3			X	X		1*A*
4	X	X			X	4*C*
5					X	1*B*, 11*C*
6	X	X	X	X		1*B*
7			X			2*D*
8	X	X		X	X	1*C*
9		X				1*B*, 3*C*, 1*D*
10		X		X		1*C*
11	X	X	X		X	2*C*
12		X			X	1*C*
13		X	X		X	1*A*

The discovery of complementary classes of mutants supports the hypothesis that determination is underlain by bistable circuits and suggests an experimental technique, totally independent of transdetermination frequencies, to measure distances between the determined states of disks in terms of the states of their hypothetical bistable circuits. Let us use each complementary pair of mutants to define a boundary dividing the disks, and posit the existence of a circuit in state 1 in all disks on one side of the boundary and state 0 on all those on the other side. Then, in any transdetermination which crosses that boundary, the posited circuit must be supposed to have changed state.

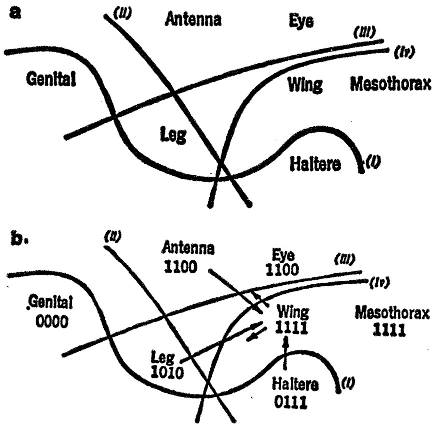

Fig. 5. (a) Four boundaries, corresponding to the four pairs of complementary mutant classes, separating imaginal disks. The hypothetical circuit corresponding to each boundary changes state only for transdeterminations crossing its own boundary. Genitalia and mesothorax were assigned to one side or another of each boundary without supporting data from mutants. (b) Use of the four boundaries and three pairs of transdetermination frequencies to assign the states of four bistable circuits to each disk.

Shearn *et al.* (*16*) did not report mutant effects on genital or mesothorax discs. For illustration, these disks are added arbitrarily to the four complementary classes: (i) (haltere, genital) and (wing, leg, antenna, eye, mesothorax); (ii) (leg, genital) and (antenna, eye, wing, haltere, mesothorax); (iii) (antenna, eye, genital) and (leg, haltere, wing, mesothorax); (iv) (haltere, wing, mesothorax) and (antenna, eye, leg, genital). Additional data suggest that the genital disk is correctly placed in pairs (i) and (ii) (*17*).

These partitions set up four distinct boundaries (Fig. 5a). Now let us posit four hypothetical circuits, each circuit changing state from 0 to 1 only for transdeterminations crossing its own boundary. In whatever manner the states of the four bistable circuits are assigned to disks (for example, Fig. 5b), so long as they are assigned consistently with the four boundaries, a haltere disk will differ from a wing disk only in the state of the circuit, circuit I, corresponding to boundary (i), but will differ from leg, antenna, and eye in the states of three circuits. Wing will differ from leg, antenna, and eye in the states of two circuits. It is legitimate, in terms of the underlying model, to make predictions about the relative frequencies of pairs of transdeterminations which cross the same boundaries, in the same direction, but in which one transdetermination crosses one or more additional boundary. Thus the boundaries predict:

Haltere to wing is more probable than haltere to eye.

Haltere to wing is more probable than haltere to antenna.

Haltere to wing is more probable than haltere to leg.

Wing to eye is more probable than haltere to eye.

Wing to antenna is more probable than haltere to antenna.

Wing to leg is more probable than haltere to leg.

Eye to wing is more probable than eye to haltere.

Antenna to wing is more probable than antenna to haltere.

Leg to wing is more probable than leg to haltere.

Wing to haltere is more probable than eye to haltere.

Wing to haltere is more probable than antenna to haltere.

Wing to haltere is more probable than leg to haltere.

Of these 12 predictions, data exist for the first 9, and all appear to be true (Fig. 4).

The model is almost certainly incomplete. It currently classifies eye and antenna as identical. Shearn and his co-workers (*16, 17*) have not saturated the mutants affecting subsets of disks, therefore additional complementary pairs of mutant classes, defining additional bistable circuits which distinguish antenna and eye, may be found. Nevertheless, using the currently defined boundaries, one can obtain 12 more predictions about relative frequencies of transdeterminations (see Table 3). Data exist for only six predictions: antenna to eye should be more probable than leg to eye, than haltere to eye, and than wing to eye. Antenna to eye should be more probable than antenna to leg, than antenna to haltere, and than antenna to wing. The first five predictions are true. The last is false (Fig, 4).

Of the 18 predictions made, one can be deduced from the truth of the others (*18*) and data are missing for three. All but two of these predictions compare transdeterminations whose frequencies either differ widely, or in which one occurs with low frequency while the second has not been observed. Therefore, despite uncertainty about exact transdetermination frequencies, those predictions which appear true are reasonably likely to be true. Fourteen tested, independent predictions are made, 13 appear to be true. I emphasize again that data for these boundaries come from mutants affecting developmental potential of subsets of disks, and are independent of data on transdetermination frequencies.

If there is a transdetermination in which only a single hypothetical circuit crosses its boundary and changes state, then, by considering whether the forward or reverse transdetermination is more probable, one can assign state 1, the more stable stationary state, to the more stable disk and to all other disks on the same side of the boundary as that disk. Since haltere to wing is more probable than the reverse, and a single hypothetical circuit, circuit I, changes state (Fig. 5a), it is assigned state 0 in the haltere and genital disks, and state 1 in the wing and the remaining disks. Of the eight possible asisgnments of the states of the remaining three circuits only one (Fig. 5b) unambiguously fulfills the condition that the transdetermination frequency from leg to wing is greater than from wing to leg, and that from antenna to wing is greater than wing to antenna (Fig. 5b).

The assignment of states of the four circuits requires the use of only three pairs of transdetermination probabilities. Once made the assignments allow nine additional independent predictions (see Table 3):

Eye to wing should be more probable than wing to eye.

Antenna to wing should be more probable than antenna to leg.

Antenna to leg should be more probable than wing to leg.

Leg to wing should be more probable than leg to eye.

Leg to wing should be more probable than leg to antenna.

Eye to wing should be more probable than eye to leg.

Eye to leg should be more probable than wing to leg.

Leg to eye should be more probable than wing to eye.

Leg to antenna should be more probable than wing to antenna.

Table 3. Predictions (T, true; F, false) generated from the model in Fig. 5. The first group of predictions may be made from the four boundaries alone. For the second group of predictions, the four circuits in each disk were assigned states, as in Fig. 5b. The third group of predictions derive from the extension of the boundaries to the mesothorax. S, status.

Predictions	S	Predictions	S
Predictions made from the four boundaries alone			
H→W > H→E	T	A→E > L→E	T
H→W > H→A	T	A→E > H→E	T
H→W > H→L	T	A→E > W→E	T
W→E > H→E	T	A→E > A→L	T
W→A > H→A	T	A→E > A→H	T
W→L > H→L	T	A→E > A→W	F
E→W > E→H	T	E→A > L→A	?
A→W > A→H	T	E→A > H→A	?
L→W > L→H	T	E→A > W→A	?
W→H > E→H	?	E→A > E→L	?
W→H > A→H	?	E→A > E→H	?
W→H > L→H	?	E→A > E→W	?
Predictions made after the four circuits are assigned states			
E→W > W→E	T	E→W > E→L	T
A→W > A→L	T	E→L > W→L	F
A→L > W→L	T	L→E > W→E	F
L→W > L→E	T	L→A > W→A	F
L→W > L→A	T		
Predictions made after extension of boundaries to mesothorax			
M→W > M→E	T	A→M > M→A	T
M→W > M→A	T	L→M > M→L	T
M→W > M→L	T	A→E > A→M	T
W→M > W→A	T	E→A > E→M	?
W→M > W→L	T	A→M > A→L	F
W→M > W→E	T	E→M > E→L	?
E→M > M→E	T	L→M > L→A	F
L→M > L→E	?	E→A > M→A	?
A→E > M→E	T	M→W > E→W	F
W→M > E→M	T	M→W > A→W	F
W→M > A→M	T	M→W > L→W	F
W→M > L→M	T		

The first six predictions appear to be true, the last three may be false, although they are not clearly so. For example, the last prediction is disconfirmed .03 to .06, one case in 30 against two cases in 31 (*4*). No predictions have been made concerning transdetermination either to or from the genital and mesothorax disks, because they were assigned to mutant classes arbitrarily.

The states assigned to all the disks generate the globally oriented transdeterminations toward mesothorax. In addition to fitting the observed inequalities, the model can fit the observed quantitative transdetermination probabilities reasonally well. Once each of the four circuits in each disk is assigned a state, there are nine adjustable continuous parameters, the two transition probabilities of each circuit, and *N*, the number of independent transdetermination trials occurring within one test implant. Data exist for 20 transdetermination probabilities (Fig. 4). The nine parameters can be chosen (Table 4) to fit the 20 observed probabilities quite closely (Table 5). As expected, for each circuit, the 0 → 1 transition is considerably more probable than the 1 → 0 transition. It is assumed that the four circuits behave independently.

One would expect that the data of Shearn *et al.* (*16*) on mutants affecting subsets of disks would provide information about the similarities and differences in the states of determination of different disks, as do data on transdetermination frequencies. The interpretation that both phenomena are underlain by bistable genetic control circuits appears to link the two types of data. Instead, Shearn's mutant data might be used to measure the distance between the states of determination of disks in terms of the number of mutants affecting each pair of disks in common. Such distance measures seem to give poorer predictions about transdetermination frequencies than those derived from the four boundaries.

The spatial arrangements of the disks in Figs. 1, 3, and 5 do not repre-

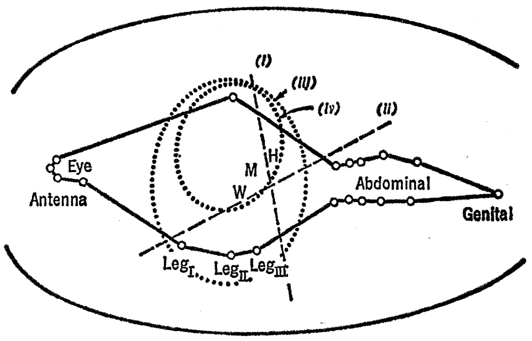

Fig. 6. The four boundaries transposed onto the fate map of the egg (*19*); *W*, wing; *M*, mesothorax; *H*, haltere. Leg_{I}, Leg_{II}, and Leg_{III} indicate fore, mid, and hind legs, respectively.

sent thier geometric relations on the fly. Because disk determination appears to occur during blastoderm formation at the end of cleavage (*1*), it is appropriate to transpose the four boundaries in Fig. 5 onto a fate map (*19*) of the egg showing the relative geometric positions of various disks (Fig. 6). Each boundary separates disks with a circuit in state 1 from disks in which the same circuit occurs in state 0. Except for the appropriate separation of the haltere, wing, legs, genital, antenna, eye, and mesothorax disks, the exact spatial location of each boundary is arbitrary. Nevertheless, it appears that boundaries (i) and (ii) each divide the embryo into two regions, anterior and posterior; boundary (iii) divides the embryo into middle versus ends; and boundary (iv) divides it into dorsal middle versus the remainder.

Projection of the four boundaries onto the fate map provides useful information. It allows prediction of the effects of Shearn's mutants on disks not yet examined. For example, abdominal disks lie on the fate map between the genital and the wing disks, and the map suggests that either state 0010 or 0000 ought to occur for abdominal disks. The map also emphasizes the important distinction between processes that need only occur once in embryogenesis for each "memory circuit" and that set up each boundary by signaling to each circuit the regions of the embryo in which state 1 or state 0 should be assumed, and processes that constitute the "memory circuit" itself which thereafter maintains the 1 or 0 state in the appropriate regions. The boundaries in Fig. 6 give clues to the geometries of the signaling processes, gradients or otherwise, which constitute the positional information interpreted by each circuit. It is of interest that the geometries of the four boundaries in Fig. 6 are simple compared to the boundaries expected by randomly choosing four pairs of complementary subsets of disks. Quite simple anterior posterior, and middle versus end gradients or waves could generate the boundaries in the model.

Table 4. Assumed probabilities of transition from state 0 to state 1 and state 1 to state 0 for the four hypothetical circuits, I to IV, chosen to fit the quantitative frequencies of transdetermination. The frequency of transdetermination from antenna, for example, antenna to wing, is measured as the number of implants containing one or more wing regions after metamorphosis. From the model, this requires the transition of state 0 to state 1 for circuits III and IV, and occurs with the probability $[P(IV_{0\to1})][P(III_{0\to1})]$. If the implant has N independent trials at transdetermination, the probability that one or more will succeed is approximately $1 - \exp\{-N [P(III_{0\to1})][P(IV_{0\to1})]\}$. Expressions of this form were used to generate the predictions in Table 5. For these calculations I assume $N = 50$.

P 0→1	Circuit	*P* 1→0
.130	I	.0001
.120	II	.056
.0923	III	.005
.170	IV	.0601

Processes with approximately these geometries occur in some higher dipterans around the time of blastoderm formation and disk determination. In *Calliphora*, as in *Drosophila*, cleavage consists in 12 nuclear mitotic divisions during which the egg remains a syncytium. By the twelfth division, nuclei have migrated to the egg cortex, and cell walls begin to separate them, creating the blastoderm. Agrell (*20*) found that in the first eight cleavages of *Calliphora* the nuclei undergo mitosis synchronously. The last four cleavages occur as subtle mitotic waves. The ninth and tenth cleavages have synchronous metaphases, but anaphase waves propagating from the anterior to posterior end of the egg. The eleventh and twelfth divisions occur as anaphase waves propagating from both ends toward the middle of the egg (Fig. 7). Whether such anaphase mitotic waves occur in *Drosophila* is not yet known.

Further Tests of the Model

The model may be tested and extended in several ways. The hypothesis that bistable circuits control disk determination can be tested further by seeing whether Shearn's complementary mutant classes extend appropriately to other disks. The extension of one boundary to include, say, mesothorax, requires appropriate behavior of two mutant classes; one must affect mesothorax, the other must not. I tentatively assigned mesothorax state "1111" in Fig. 5b to account for the transdeterminations oriented toward it. This prediction, extending all four boundaries, requires that each mutant class that affects wing will also affect mesothorax. Of the 12 mutants comprising the eight mutant classes which are used to form boundaries, 11 behave as predicted (*17*). It appears that boundaries (ii), (iii), and (iv) extend appropriately to mesothorax. Boundary (i) appears not to extend to mesothorax. This is a failure, but it should be noted that the ability to predict disjoint complementary mutant classes rests on the stringent assumption that the disruption of one stationary state of a bistable circuit will disrupt every disk in which that circuit state occurs.

The probability that 13 mutant classes affecting 13 of the $2^5 - 2 = 30$ subsets of the first five disks considered will have four or more complementary pairs is .19. Extension of Shearn's

Table 5. Fit between predicted and observed transdetermination frequencies. The first eight observed frequencies were used to adjust the parameters in Table 4. The precise transition frequencies for each circuit in Table 4 should not be given much credence because they depend upon *N* which may not be independently measurable.

Transdetermination	Observed	Predicted
G→A	.54	.54
G→L	.46	.46
L→W	.64	.64
A→L	.23	.23
L→A	.03	.03
G→W	.035	.012
W→L	.16	.16
W→H	.00	.001
A→W	.57	.55
W→A	.06	.015
H→W	.99	.99
H→L	.00	.019
H→A	.00	< .001
H→G	.00	< .001
A→G	.00	< .003
L→G	.00	< .0003
G→H	.00	.094
L→H	.00	< .0001
A→H	.00	< .0001
W→G	.00	< .0001

mutant classes to the mesothorax reveals three complementary pairs. The probability that 13 mutant classes affecting 13 of the $2^6 - 2 = 62$ subsets of six disks have three or more complementary pairs is .084. It seems reasonably unlikely that the occurrence of complementary mutant classes is due to chance (*21*).

Extension of the boundaries to the mesothorax, and the assignment of states to three of its circuits (?111), generates 23 more predictions about relative transdetermination frequencies. Data exist for 19 predictions, of which 14 appear to be true (Table 3).

Of the 55 independent predictions listed in Table 3, 33 appear to be true, 9 false, and 13 unknown. Assessment of the unestablished predictions would test the model. Eight of these predictions would be true if eye transdetermined to antenna at an appreciable frequency. However, these eight predictions may not be meaningful, for the eye-antenna disk is a single structure. The half that determines eye alone can regenerate antenna derivatives, although the half that determines antenna appears not to regenerate eye derivatives (*22*). It is likely, therefore, that the eye disk will appear to "transdetermine" to antenna, but whether such alterations in determination would represent transdetermination, or pattern regulation within one disk, or how the two processes are related, is unclear. Three of the remaining five unestablished predictions assert that transdetermination from wing to haltere is more frequent than eye, antenna, or leg to haltere. No such transdetermination has been observed. In view of the established transdetermination sequences (see Fig. 1) in which haltere transdetermines only to wing, it would be surprising if these predictions proved false, for that would require transdetermination from antenna, eye, or leg to haltere to be more frequent than wing to haltere. It may be significant that homeotic mutants altering wing to haltere are known [contrabithorax (*23*)], but none are known that alter eye, antenna, or leg, to haltere; however the relation between homeotic mutants and transdetermination is unclear.

Nine of the 55 predictions appear false, therefore the model cannot be correct in detail. Seven of the false predicted inequalities in transdetermination frequencies may be due to the obvious incompleteness of the model, which currently classifies eye and antenna as identical, and wing and mesothorax as identical. In addition to false predicted inequalities, these identities predict that transdeterminations to and from eye should equal those to and from antenna, while those concerning wing and mesothorax should be equal. Assessment of equal frequency is difficult, but at least 5 of 14 predicted transdetermination frequencies (Fig. 4) are false.

The ad hoc assumption that two additional circuits distinguish eye from antenna, and wing from mesothorax, would remove seven of the nine false inequality predictions and all the equality predictions. It should be possible to obtain evidence for such additional circuits; classes of mutants affecting subsets of disks have not been saturated, and pairs of mutants affecting other complementary subsets of disks may be found.

If bistable circuits underlie each complementary pair of mutants, it should be possible to discover mutations which alter the relative stabilities of the two stationary states, but otherwise leave those states normal. A mutant destabilizing one stationary state should increase the transition frequency from that state to the other stationary state. The effect should be coordinated increased transdetermination frequencies from all disks in which the destabilized state of that circuit occurs toward all those disks in which the unaffected state of that circuit occurs. If pairs of such mutants, affecting complementary subsets of disks, could be found, it would give strong indication of a circuit which actually switches between two alternative states. This would be stronger evidence for bistable circuits than pairs of mutants which merely block development in complementary subsets of disks. Were the complementary pairs affecting transdetermination frequencies to coincide with the four complementary pairs found by Shearn *et al.* (*16*), the current boundaries would be further supported (*24*).

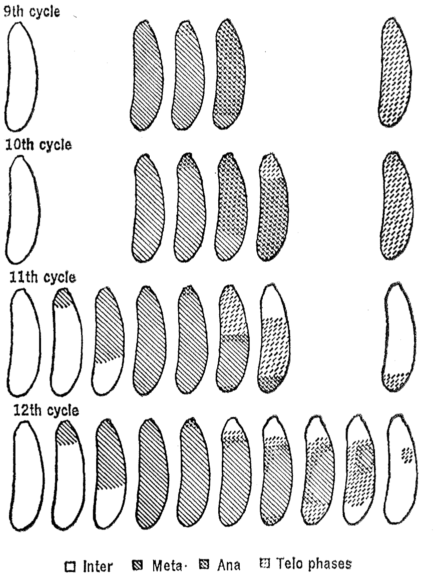

Fig. 7. Mitotic waves in the ninth through twelfth cleavage divisions in *Calliphora*. The ninth and tenth divisions exhibit anterior posterior anaphase waves. The eleventh and twelfth divisions show ends to middle anaphase waves. [After Agrell (*20*)]

Some Homeotic Mutants May Affect Circuit Stability

Homeotic mutants result in flies that have a normal organ or part of an organ in an abnormal location. For example, the aristapedia mutant grows leg parts in place of the arista (*7*). At least two quite distinct mechanisms might account for homeosis. The "memory circuits" that maintain the determined state in each disk might be faulty and unable to maintain a particular state. This hypothesis corresponds to the suggestion above that mutants might destabilize one stationary state of some bistable circuit. If so, that instability should be present at all times after determination and before metamorphosis. Alternatively, the "memory circuits" may be entirely normal, but the signaling processes that initially set the four circuits to the appropriate state in each disk might be faulty. Such mutants would be expected to act only at the time of determination and not throughout larval life. In addition, such mutants should not affect the stability of the "memory circuits."

Some homeotic mutants appear to belong to the first class. When heterozygotes of some recessive homeotic mutants are irradiated late in larval development to achieve somatic crossing over, the homozygous homeotic clone exhibits the homeotic abnormality after metamorphosis (*23, 25*). Since the alteration occurs after the supposed time of determination, the mutant presumably affects the circuits maintaining determination, not the initial signaling processes. This suggests that at least some homeotic mutants may affect the stability of the circuits underlying determination, and the possibility that they cause coordinated increased transdetermination from one subset of disks to a complementary subset should be examined. The hypothesis that bistable control circuits underlie determination does not predict that all homeotic mutants will result in a coordinated increase in transdetermination frequency, because some homeotic mutants may be due to erroneous initial signaling to circuits, or to other mechanisms, or circuits may not be entirely independent of one another (*24*).

There are at least two homeotic mutants that show coordinated alterations from more than one disk to more than one target disk. The patterns of their alterations seem to fit the four boundaries in the model. In one mutant found by Shearn *et al.* (*16*), haltere disks implanted into metamorphosing larvae often develop as wings, while genital disks often develop as either antenna or leg patches. This mutant was used in part to define boundary (i) in Fig. 5, hence the model interprets, but does not predict, this coordinated increased transdetermination frequency as a destabilizing of the 0 state of circuit I.

It has recently been shown (*26*) that the tumorous head mutant, ***tuh***, is a homeotic mutant in which genital structures appear in place of antenna disk derivatives in the adult. Were this due to a destabilization of state 1 of circuit II in Fig. 5b, the model would predict coordinated increased transdeterminations from antenna to genitalia and also to leg. Furthermore, transition to leg should be more common than to genitalia. Leg structures do occur, and more commonly than genital structures, in place of antenna derivatives in adult *tuh* flies. Since antenna and eye disks are classified as having identical determination states, this incomplete model predicts that *tuh* should destabilize the eye disk and increase transdetermination toward leg and genitalia. In fact, the eye disk is destabilized, but it transforms to abdominal tergites which, according to Fig. 6, should be very similar or identical to the genital disks in the four circuits so far specified. The model also predicts that transdetermination frequency from wing to leg should increase. Were its penetrance and expressivity high enough, it might be seen in intact *tuh* flies. Postlethwait (*27*) has not been able to confirm this prediction on adult *tuh* flies, and experiments are being designed to test the predicted increased transdetermination frequencies on the different disks.

The model presented in Fig. 5 depends heavily on the discovery of pairs of mutants affecting complementary subsets of disks, and the interpretation placed upon them. Both data and interpretation are subject to criticism. First, in asserting that a mutant affects only a subset of disks, Shearn *et al.* (*16*) were asserting that it did not affect other disks. Such an assertion requires the unavoidably subjective judgment that the unaffected disks were fully normal. Second, I interpret Shearn's mutants, which are lethal in the late larval stage and alter normal development of some disks upon test implantation in metamorphosing larvae, as being mutants that affect a process correlated with the determined state. Do they? One mutant found by Shearn is homeotic and leads haltere disks to develop as wings, genital disks to develop as antenna and legs. This mutant, used in boundary (i), clearly affects determination. However, not all mutants affecting determination would be expected to change one normal determined state to another. The only criterion of determination currently available is operational: a disk is determined to become wing if, upon metamorphosis, it becomes wing. If a mutant leads to abnormal development of specific disks at metamorphosis, it is not clear how one could prove that it affects determination.

Furthermore, as discussed earlier, it is too stringent to insist that the mutants affect determination itself. Suppose each state of any memory circuit has specific metabolic requirements, or primes specific additional genes to be activated by ecdysone during metamorphosis. Mutants not affecting determination itself, but such additional processes specifically associated with only one state of each "memory circuit," would still be expected to fall into pairs affecting complementary subsets of disks if the circuits are bistable. But even for this weaker assumption, independent evidence, beyond the predictions based on the four boundaries, that Shearn's complementary mutant classes affect processes related to determination is not yet available.

Conclusion

The facts that imaginal disk determination persists for long periods, and that transdetermination occurs in specific sequences, strongly suggest a number of general conclusions. The determined state in any disk must consist in a self-maintained stationary state of one or many "memory" entities, each of which has a number of distinct alternative stationary states. If more than one "memory" entity is involved, the determined state in each disk is characterized by a unique combination, or small number of combinations, of states of the "memory circuits." This yields both a natural distance measure between the states of determination of any two disks, the number of circuits required to change state to alter from one to the other state of determination, and an explanation for sequences of transdetermination.

The global orientation of transdetermination toward mesothorax, the pairs of mutants affecting complementary subsets of disks, and the successful predictions of relative transdetermination frequencies based on such mutants being interpreted as signs of bistable circuits, suggest determination is underlain by "memory circuits" with two stationary states, one considerably more stable than the other. Elsewhere (*28*) I have tried to show that control circuits with precisely these properties, as exhibited by the C_1 *tof* loop in bacteriophage lambda, very probably occur in higher organisms.

Although the detailed model presented herein is at best incomplete, it indicates that one should be able to obtain answers to a number of questions about the underlying circuits without yet knowing their detailed microscopic construction. For example, we should be able to determine how many circuits there are; whether the more stable or less stable state of each circuit occurs in each disk; what the quantitative relative stabilities are of the two states of each circuit; and what the geometries are of the boundaries separating the two states of each circuit in the early embryo.

It might be argued that such an abstract approach that concerns the macroscopic behavior of the circuits does not lead directly to their molecular construction and does not warrant the work required. I think that such an argument would be unjustified. It is not unlikely that the successful decomposition of the determined state of each disk into a combination of entities, each of which behaves largely as an independent unit, and the discovery of the macroscopic patterns of each unit's behavior among the imaginal disks, will substantially aid our discovery and recognition of the correct molecular circuitry.

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29. I am grateful to Drs. J. Cowan, A. Garen, R. Nothiger, E. Hadorn, W. Gehring, A. Shearn, and J. Postlethwait for their critical comments, and to the last four for kindly sharing unpublished data. I thank Dr. Anthony Dursten for pointing out the similarity in the geometries of the mitotic waves reported by Agrell and the boundaries in Fig. 6. This research was supported in part by the Sloan Foundation.

On-Line Services in Medicine and Beyond

A national and international bibliographic information network for science and technology is now evolving.

Davis B. McCarn and Joseph Leiter

On 29 October 1971, the National Library of Medicine (NLM) initiated a nationwide, on-line, bibliographic retrieval system as a general service for the biomedical community. This service, called MEDLINE, now allows almost instantaneous, interactive searching of over 400,000 citations from the world's biomedical serial literature. After a year, the service was supporting an average of 25 simultaneous users, 43 hours per week; in October 1972, 10,605 searches were processed—a rate of nearly 140,000 searches per year. Service is provided through a data communication network that allows access through a local dataphone call in any of 40 major metropolitan areas across the nation. Over 120 institutions with over 200 terminals are using the service. The communications network also has a node in Paris and is being used regularly by the French MEDLARS center for a trial period. The Canadian MEDLARS center is a regular part of the network. The MEDLINE data base is also operated from a computer in Sweden, and access is provided by remote terminals in six locations in Sweden through regular telephone lines. MEDLINE is the first generally accessible, on-line, interactive information service. It constitutes the first national and international telecommunications-based science information network.

Background

Interactive systems for timesharing were developed and demonstrated successfully in 1963 by two parallel projects, one at the Massachusetts Institute of Technology and the other at the System Development Corporation, both funded by the Advanced Research Projects Agency of the Department of Defense. Project MAC at Massachusetts Institute of Technology concen-

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