Table 2. Mean of extra bristle numbers in females bearing different combinations of *Hw* and *Hw*$^+$ alleles with line E and *Hw* stock polygenic backgrounds. Interaction effect between the *Hw* allele and the line E polygenic background.

		Genetic background	
		E line	Hw stock
		Hw (E)	Hw (+)
Genotype at the Hw locus	Hw/Hw$^+$	6.26 ± 0.23	1.86 ± 0.15
		Hw$^+$ (E)	
	Hw$^+$/Hw$^+$	1.44 ± 0.18	

Interaction effect $(\bar{X}_{Hw(E)} - \bar{X}_{Hw(+)}\bar{X}_{Hw^+(E)}) = 2.96 \pm 0.10$
$z = 9.16 \quad p \ll 0.001$

If the line E genes improve the chaetogen synthesis, the introduction of the *brs* allele (which, as mentioned before, we have assumed to interfere with the chaetogen synthesis) in a line E background should suppress extra bristles before reducing the size of normal ones. The relatively independent behavior of normal and extra bristles can be accounted for only if the modified variable is the cells' response to positional information.

If this is so, the *Hw* effects and those of the E background will not be additives since the *Hw* allele increases the chaetogen synthesis[13]. Females from a *Hw* stock were crossed with line E males and, starting from the F_1 females, five successive backcrosses of *Hw*/*Hw*$^+$ females to line E males were practised in order to obtain females bearing the *Hw*/*Hw*$^+$ or the *Hw*$^+$/*Hw*$^+$ genotype in the same E background. By comparing them with females from the *Hw* original stock (*Hw*/*Hw*$^+$ because of the recessive lethality of *Hw*), an important interaction between the *Hw* allele and the E polygenic background (table 2) was found, which reinforced the hypothesis that selection for extra bristles has modified the cell response to positional information.

The Richelle and Ghysen model has shown to be a very useful tool for interpreting the mechanisms that selection has altered to produce the phenotypes selected in lines E and S. The main conclusion of this work is that there is genetic variability for the interpretation of positional information and, therefore, it can be modified by selection. Confirmation of that hypothesis would involve the generation of somatic mosaicism, but this will be very difficult to perform because of the small effect of the located genes and the importance of their interaction with the rest of the polygenic background.

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Sclerotial and low aflatoxigenic morphological variants from haploid and diploid *Aspergillus parasiticus*[1]

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Summary. Serial transfer of mycelial macerates of a wild type, haploid, aflatoxigenic strain of *Aspergillus parasiticus* in a defined liquid medium resulted in the production of three new morphological classes: a sclerotial form with high aflatoxin production, and two variant forms (*fan* and *fluff*) with lowered sporulation, no sclerotia, and attenuated levels of aflatoxin. A genetically marked diploid containing mutant markers for aflatoxin pathway intermediates yielded the same three morphological classes upon serial transfer of macerated mycelia. When these diploid variants were treated with a haploidization agent, and the phenotypes of the resultant segregants scored, a low frequency of colonies producing aflatoxin pathway intermediates was recovered. These genetic data indicate that the structural genes for the aflatoxin pathway are present but somehow attenuated in the *fan* and *fluff* strains.
Key words. Aflatoxin; *Aspergillus parasiticus;* sclerotia; mycelial maceration; strain degeneration.

Aflatoxins are toxic, carcinogenic secondary metabolites produced via the polyketide pathway by the common molds *Aspergillus flavus* and *A. parasiticus*. Among agricultural isolates of these species there is wide variation in toxigenicity. Some strains produce no detectable aflatoxins, while producers vary several-fold in the amount of toxin synthesized. Moreover, high producers may become low producers after several transfers in the laboratory[3,4]. Experimental induction of lowered or lost aflatoxin-production has been reported after subjecting *A. flavus* to several generations of growth in medium containing barium ions[5]; *A. parasiticus* in a liquid defined medium that suppresses sporulation[6,7]; and both *A. flavus* and *A. parasiticus* to successive transfers on crushed wheat[8]. Experiments on the genetic basis of this variability in aflatoxin-producing ability are difficult to formulate because neither *A. flavus* nor *A. parasiticus* has a known sexual stage and crosses must be established using marked mutants through the parasexual cycle[9,10]. Certain mutants with brightly colored mycelia accumulate anthraquinones which are known precursors of aflatoxin biosynthesis[11]. The fortuitous association of mycelial pigmentation and aflatoxin pathway intermediates allows visual scoring of the aflatoxin pathway in mutants bearing these markers. We have synthesized a parasexual diploid between a norsolorinic acid-accumulating (red) and a versicolorin A-accumulating (yellow) mutant[12] and used this diploid, as well as a wild type, highly aflatoxingenic haploid strain, to extend studies on the serial transfer of mycelial macerates in liquid defined medium

Materials and methods. The wild type haploid strain of *A. parasiticus* was designated SU-1 (NRRL 5862) and its lineage was described by Mayne et al.[4]. The diploid strain (DIP-8) contained contrasting spore color (*wh*-white; *br*-brown), auxotrophic (*lys*-lysine; *ser*-serine) and mycelial color (*nor*-norsolorinic acid; red;

ver-versicolorin A, yellow) markers; its genotype was designated *br-1 nor-1 lys-5/wh-1 ver-1 ser-1*. The isolation of mutants and the synthesis of this diploid were described by Bennett[12]. All cultures were incubated at 30 °C in the dark.

The complete medium (CM) was potato dextrose agar supplemented with 0.5% yeast extract. For isolation of haploid segregants from the diploid, para-fluorophenyl alanine (FPA) was added to CM (200 mg/l) and segregants were selected as described previously[12]. The solid minimal medium (MM) was Czapeks solution agar. For identifying the phenotype of auxotrophic segregants, Czapeks agar was supplemented with lysine and/or serine (both 250 mg/ml). The liquid defined medium for serial transfer experiments, for dry weights, and for assay of aflatoxin production was formulated by Adye and Mateles[13]. The quick screening agar for putative plate assays of aflatoxin-producing ability (APA) was formulated by Hara et al.[14]. The original inocula for serial transfer experiments were prepared by flooding stock cultures of SU-1 grown on CM, or DIP-8 grown on MM, with 0.005% Tween 80 to obtain a dense conidial suspension. This spore suspension was used to inoculate 100 ml AM which was grown in shaker culture for 3 days and to obtain a viable count of colony forming units (CFU). After 3 days, the AM-grown cultures had formed mycelial pellets. The entire contents of the flask consisting of these mycelial pellets and the culture medium were poured into a Waring Blendor and blended at high speed for 1 min. One ml of the resultant mycelial macerate was inoculated into a fresh flask containing 100 ml AM; another ml was diluted appropriately and plated on CM in order to obtain a viable count of CFU's. Two parallel series of SU-1 (HAP1 and HAP2) were established and continued for 5 transfers; one series of DIP8 was established and continued for 8 transfers. Representative isolates of resultant morphological variants were grown on CM or APA for 1 week at 30 °C in the dark. The CM-grown cultures were observed for cultural characteristics. The APA cultures were inverted and viewed under long wave ultra-violet light. Blue fluorescence on APA was a putative assay for aflatoxin production; absence of fluorescence a putative assay for lack of aflatoxin production.

For dry weights and aflatoxin assays triplicate cultures were grown in AM for one week in shaker culture. For dry weights, mycelia were rinsed with deionized water and dried overnight in an oven at 70 °C. For aflatoxins, the mycelia and culture filtrates were blended with acetone in a Waring blendor and the resultant slurry filtered through a Buchner funnel. The acetone extract was adjusted with water to approximately 30% acetone and extracted twice with chloroform in a separatory funnel. The chloroform extracts were concentrated and the assayed on Adsorbosil 1 silica gel plates by thin layer chromatography in ether: methanol: water (96:3:1, v/v/v). Aflatoxins were densitometrically quantitated according to the method of Pons et al.[15] using a Schoeffel SD 3000 recording densitometer.

Results and discussion. The results of the serial mycelial maceration experiments are presented in table 1. Four morphological variants were observed and a summary of their general morphologies, culture characteristics, and aflatoxigenicity are given in table 2. All four variants exhibited fundamental characteristics diagnostic of the species *A. parasiticus* including: small vesicles (25–35 µm diam.), uniform conidium size (mostly 5.0–5.5 µm diam.), metulae lacking, color of conidia darkening with age, and enhanced growth at 37 °C[16].

The sclerotial form most closely resembled wild type in morphology and toxigenicity. It was the first variant to appear, becoming visible in the DIP8 series in first transfer. The largest number of sclerotial types was seen at the fourth transfer; subsequently their frequency declined. No sclerotial variants were seen after five transfers of the haploid series or seven transfers of the diploid series.

Taxonomists give conflicting accounts of sclerotial production within *A. parasiticus*. Raper and Fennell[17] state 'No sclerotia or cleistothecia reported'; Christensen[18] considers that presence or absence of sclerotia is not a valid species criterion in the *A. flavus* group; and Murakami et al.[19] report 'sclerotia sometimes produced'. The lability of the sclerotial phenotype within one strain in this controlled study makes the lack of agreement among taxonomists studying culture collection strains more understandable.

Table 1. Production of morphological variants by serial transfer of mycelial macerates of haploid and diploid *Aspergillus parasiticus* in defined liquid medium[a]

Transfer (3-day-intervals)	Culture series[b]	Colony Forming Units (CFU/ml × 10^4)				Percent change from wild type
		Wild type[c]	Sclerotial[c]	Fan[c]	Fluff[c]	
Inoculum	HAP1	137	0	0	0	0
	HAP2	137	0	0	0	0
	DIP8	200	0	0	0	0
First	HAP1	31.5	0	0	0	0
	HAP2	27.6	0	0	0	0
	DIP8	16.0	0.5	0	0	3.0
Second	HAP1	19.6	0	0	0	0
	HAP2	26.5	0	0	0	0
	DIP8	9.0	0.3	0	0	3.2
Third	HAP1	19.5	1.0	0.4	0.3	8.0
	HAP2	9.8	0	1.0	0	9.3
	DIP8	10.0	1.7	0	0	14.5
Fourth	HAP1	23.0	3.0	0	5.0	25.8
	HAP2	19.8	1.5	3.8	0	21.1
	DIP8	9.7	8.7	0	0.7	49.2
Fifth	HAP1	26.0	0	0	28.5	52.4
	HAP2	1.8	0	45.8	0	95.8
	DIP8	4.7	2.7	0	0.3	38.9
Sixth	DIP8	3.3	2.0	1.7	1.0	58.8
Seventh	DIP8	0	0	9.2	10.2	100
Eighth	DIP8	0	0	17.5	23.4	100

[a] Defined medium formulated by Adye and Mateles[12]; [b] HAP1 and HAP2 are parallel series from the same original inoculum of haploid wild type SU-1, DIP8 is a separate series derived from a parasexual diploid strain[11]; [c] Detailed descriptions of morphology given in accompanying text and table 2.

Table 2. The cultural characteristics, dry weight, fluorescence and aflatoxin-producing ability of four morphological variants of *Aspergillus parasiticus*

Cultural characteristics of morphological variants[a]	Ploidy[b]	Mycelial dry weight (mg/100 ml AM)[b]	Fluorescence on APA[c]	Aflatoxin (μg/100 ml AM[b]) B_1	G_1	Total
Control ('wild type'):	HAPLOID	1180	+ +	1975	275	2250
Abundant dark green spores; buff color on reserve of plate; aerial mycelium only with aging; few sclerotia (< 25) per petri plate	DIPLOID[d]	1310	±	103	4	107
Sclerotial:	HAPLOID	1054	+ +	687	192	879
Almost identical to wild type except numerous sclerotia (> 200) per plate	DIPLOID[d]	–	±	–	–	–
Fan:	HAPLOID	1015	–	0.5	Tr	0.5
Abundant dark green spores in center of colony, scant sporulation at perimeter; buff colored reverse with fan-like ridges; no sclerotia	DIPLOID	1210	–	0.2	ND	0.2
Fluff:	HAPLOID	1300	–	367	5.4	372.4
Scant light green spores, abundant cottony aerial mycelium; buff color on reverse with heavy furrows; no sclerotia	DIPLOID[d]	880	–	1.2	ND	1.2

[a] Phenotype scored on potato dextrose agar + 0.5% yeast extract; [b] AM = Adye and Mateles medium[12]; [c] APA = Aflatoxin producing ability medium[13]; [d] haploid and diploid variants looked similar except that the diploid tended to form sectors displaying mutant spore color and mycelial color markers.

The haploid control and sclerotial forms produced intense blue fluorescence indicative of aflatoxin production on APA medium, and densitometric analysis confirmed high (> 800 μg/100 ml AM) aflatoxin production. The control diploid strain produced low fluorescence and moderate levels (approximately 100 μg/100 ml AM) aflatoxin. The sclerotial variants of the diploid gave similar fluorescence to the diploid control, but aflatoxins were not assayed quantitatively. Haploid and diploid isolates of the *fan* and *fluff* phenotype both exhibited lowered sporulation, altered colony characteristics, and attenuated aflatoxigenicity. The two types could easily be distinguished because the *fan* variants yielded flat growth with heavy sporulation in the center of the colony but only scant sporulation at the edges, while the *fluff* variants produced abundant cottony, aerial mycelium, and few spores. The *fan* variants first appeared in the third transfer of the haploid cultures and the sixth transfer of DIP8; the *fluff* variants were first observed after the third transfer of HAP1 and after the fourth transfer of DIP8.

No isolates of *fan* or *fluff* produced fluorescence on APA agar. Extremely low levels of aflatoxins (0.2–0.5 μg/100 ml mlAM) were detected by densitomeric analyses of haploid and diploid *fan* variants (table 2). Although the *fluff* variants did not fluoresce on APA agar, significant aflatoxin levels were detected densitometrically. Individual isolates from HAP1 ranged from 120–420 μg aflatoxin/100 ml AM. The DIP8 *fluff* isolates yielded low levels (< 2 μg/aflatoxin/100 ml AM). These quantitative analyses do not support our preliminary reports that *fan* and *fluff* are nonaflatoxigenic[7,20,21].

In order to learn more about the expression of the aflatoxin pathway in the *fan* and *fluff* phenotypes, the control, *fan*, and *fluff* variants of DIP8 were treated with the haploidization agent FPA and fast growing sectors were scored for spore color, mycelial color, and nutritional requirement. These results are presented in table 3. There was extreme selection against recovery of auxotrophic markers for all three morphological classes. Of a total of 1341 segregants analyzed, only 13 displayed nutritional deficiencies. Similar selection against auxotrophic markers on media with FPA has been reported in previous parasexual analyses in *A. parasiticus*[12,22]. Most fast growing sectors had green spores, and distinct classes of dark green and light green were distinguished for the control and *fan*, but not *fluff*. Among *fan* segregants there were fewer brown-spored than white-spored isolates, and only one brown-spored isolate was recovered from *fluff*. Mycelial pigmentation reflects either the production of norsolorinic acid or versicolorin A. Both these polyhydroxyanthraquinones are known intermediates in the aflatoxin pathway[11]. For the control DIP8, both pigmented (248) and unpigmented (243) segregants were recovered in almost equal frequency. However, only 5 of 545 *fan* segregants were pigmented and only 2 of 305 *fluff* segregants were pigmented. Since sectors bearing these markers are recovered in high frequency for the control, it seems unlikely that selection against pigmented sectors is the explanation for the low frequency of this class from *fan* and *fluff*. Moreover, these rare, pigmented segregants from *fan* and *fluff* were unstable, segregating unpigmented sectors. Somatic instability is frequently a diagnostic criterion for cytoplasmic genes[23,24], but in a previous

Table 3. Numbers of segregants from control, *fan* and *fluff* variants of DIP-8 (*br-1 nor-1 lys-5/wh-1 ver-1 ser-1*[a]) treated with para-fluorophenyl alanine

Mycelial type	Spore color	Prototrophic Unpigmented	Prototrophic Pigmented[c]	Auxotrophic[b] Unpigmented	Auxotrophic[b] Pigmented[c]	Total
Control	Brown	25	22	0	2	49
	White	32	1	4	1	38
	Light green	105	159	0	0	264
	Dark green	82	58	0	0	140
	Total	244	240	4	3	491
Fan	Brown	36	1	0	0	37
	White	100	4	2	0	106
	Light green	349	0	1	0	350
	Dark green	50	0	2	0	52
	Total	535	5	5	0	545
Fluff	Brown	1	0	0	0	1
	White	108	0	1	0	109
	Green	195	1	0	0	195
	Total	304	1	1	0	305
Total		1083	246	10	3	1341

[a] The symbols for gene markers are described in the text; [b] lysineless (6), serineless (6), double auxotrophs (2); [c] either red (norsolorinic acid) or yellow (versicolorin A).

study we were unable to separate the *fan* and *fluff* phenotypes from nuclear markers in a heterokaryon test[20].

The high frequency with which *fan* and *fluff* variants are isolated, and the pleiotrophic nature of their respective phenotypes, is characteristic of a common, but poorly understood phenomenon in filamentous fungi variously called 'strain degeneration' and 'vegetative instability'. Aged cultures, and strains maintained for a long time in the laboratory with frequent subculturing, are particularly prone to such 'degeneration' which may yield visible sectors on agar medium, and which may manifest itself by changes in morphology, attenuated virulence and/or loss of secondary metabolite production. The early antibiotics literature is filled with references to strain degeneration in *Penicillium notatum* and *P. chrysogenum*[24-28].

Somatic instability in molds is attributed to mutation, heterokaryosis ('the dual phenomenon'), physiological adaptation, cytoplasmic heterogeneity, and anamolous chromosome mechanics[24,29-32]. In Actinomycetes, strain instability is associated with plasmid loss, gene amplification, and gene transpositions[33]. Genetic instability has been demonstrated in *A. nidulans* among transformants, which exhibit a wide variety of morphological phenotypes with successive subculture of individual transformants[34].

We find genetic transposition an attractive model to describe the anomalous behavior of our morphological variants in *A. parasiticus*. Whatever their origin, their high frequency from mycelial macerates would recommend against the practice of blending mycelia in an attempt to get a uniform inoculum, as has been adopted by certain workers studying versicolorin[35] and aflatoxin[36] metabolism.

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Monogenic inheritance of cyclodiene insecticide resistance in mosquitofish, *Gambusia affinis*

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Summary. Certain populations of the mosquitofish, *Gambusia affinis*, developed high levels of resistance to endrin and other cyclodiene insecticides as a result of inadvertent exposure to agricultural sprays. Genetic crossing studies show that endrin resistance is inherited as a single, autosomal, intermediate gene.
Key words. Insecticide resistance; inheritance; mosquitofish; cyclodienes; endrin; picrotoxinin.

Certain populations of the mosquitofish, *Gambusia affinis*, developed high levels of resistance to endrin and other cyclodiene insecticides as a result of inadvertent exposure to agricultural sprays. The similarities of cross-resistance patterns between insects and mosquitofish suggested that the inheritance of resistance in mosquitofish might be similar to that in insects. Numerous genetic studies of cyclodiene resistance in insects show that it is nearly always inherited as a single allele of intermediate expression (often referred to as 'semi-dominant')[1,2]. By intermediate we mean that the phenotype of the heterozygote (RS) is almost exactly the logarithmic mean of the LC_{50}'s of the homozygous resistant (RR) and susceptible (SS) strains[3]. We report here that cyclodiene resistance in *Gambusia* is a monofactorial autosomal intermediate trait, as demonstrated by crossing experiments.

Cyclodiene insecticides, including endrin, dieldrin, and toxaphene, were widely used for control of agricultural and medical pests from the early 1950's until the late 1970's, when their use