AZYGOTIC RATIOS IN HABROBRACON*

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There are gradually accumulating in genetic literature, data on organisms of widely diverse groups which may be known as azygotes. Such organisms arise from cells, unfertilized eggs in animals (parthenogenesis) or spores in plants, resulting from complete reduction in meiosis. Since it is now well known that reduction may occur in part in the first, in part in the second maturation division, even as regards a single chromosome tetrad, it is clear that two divisions are necessary to completely reduce the nucleus. This reduced nucleus is usually thought of as haploid but such is not necessarily the case. In tetraploid plants diploid spores result, while triploid females of Drosophila produce a certain proportion of diploid or otherwise not completely reduced eggs. Aside from such cases as polyploidy in which two divisions are not sufficient to effect complete reduction, there is increasing evidence for "regulation" of chromosome number following haploid parthenogenesis. The organism developing from an unfertilized egg or spore that has undergone complete reduction may be called an azygote, regardless of whether it is haploid or diploid. If regulation occurs the diploid organism becomes homozygous. It cannot be heterozygous except as a result of mutation. Organisms originating from diploid parthenogenesis may retain more or less of the heterozygosis of their mother and are therefore not azygous.

Geneticists are interested in determining gametic (or spore) ratios. Azygotes from heterozygous mothers, if obtained in numbers large enough to minimize deviations due to sampling, approximate these ratios with the following limitations:

(1) Masking effects due to an environmental condition or to another genetic factor.

(2) Overlapping of one class with another.

(3) Viability differences caused by one or more of the pairs of allelomorphs under consideration.

(4) Disproportion in viability differences when various factors are combined.

(5) Lethal and semilethal factors linked with one or more of the genes in question.

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Subject to the above limitations zygotic ratios resulting from the cross of the heterozygote to the recessive approximate gametic ratios except that (5) linked lethal or semilethal factors have comparatively little influence. If the recessive stock be bred true, there is relatively little chance that it will contain lethals similar to any that may be linked with the heterozygous factors being tested.

In the case of sex linkage, linked lethal genes are more likely to cause deviations among the offspring of the digametic sex. Even in male Drosophila, however, it is possible that the Y chromosome may have some protective influence. In species with X-O male or Z-O female, the diagametic individuals are comparable with azygotes as far as sex-linked factors are concerned.

In males of Habrobracon all chromosomes are subject to this lethal selection, lacking protection from dominant allelomorphs. Review and discussion of azygotic ratios in other forms will be deferred until after the presentation of data on azygotic ratios in Habrobracon.

The present paper reports summaries of data which have been accumulated in recent years, largely resulting from tests for linkage made by graduate students associated with the UNIVERSITY OF PITTSBURGH. The authors wish especially to mention the work of MILTON FRANKLIN STANCATI, NEITA C. BOSTIAN, C. H. BOSTIAN, PAUL R. DAVID, P. J. WALTER, HELEN CASHDOLLAR, GEORGE D. SNELL, MAGNHILD M. TORVIK, KATHRYN A. GILMORE, and RAYMOND J. GREB.

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TECHNIQUE AND MATERIALS

Crosses are made involving two or more mutant types and F_1 females are isolated and bred as virgins. Counts of the broods furnish the ratios of males here reported, as well as irregular types (mosaics, impaternate females) occurring infrequently and disregarded in the present summaries. When two mutant types are female sterile, the female heterozygous for one is crossed with the male of the other, in which case only half of the F_2 fraternities are of value. The mutant factors may be introduced from different parents (repulsion tests, $a \times b$) or the double mutant type may be crossed with wild-type (coupling tests $a.b \times +$). It is advisable to make these reverse crosses to check deviations due to viability differences, but thus far most tests have been of the repulsion type as this is the more convenient way to test new factors. No consistent differences have been found between F_2 fraternities resulting from reciprocal crosses (a ♀ × b ♂ and b ♀ × a ♂ in case of repulsion tests or + ♀ × $a.b$ ♂ and $a.b$ ♀ × + ♂ in case of coupling tests).

Tests of the following mutant factors are here reported: Bar eye; Kidney, *k* (eyes) and its allelomorphs, small, k^s, and extreme-small, k^e; Orange, *o* (eye) and its allelomorph, ivory, o^i; Defective, *d* (R_4 wing vein); Wrinkled, *w* (wings); Reduced, *r* (wings); Miniature, *m* (body); Fused, *f* (segments of tarsi and antennae); Cantaloup, *c* (eyes); Long, *l* (antennae and wings); Wavy, *wa* (wings); Broad, *br* (thorax); Eyeless, *el*; Shot-veins, *sv* (wings); Maroon, *ma* (eyes); Yellow, *Y* (antennae); Semilong, *sl* (antennae and wings); Tapering, *ta* (antennae); Narrow, *n* (wings); Spread, *sp* (wings); Vestigial, *v* (wings); Beaded, *b* (legs and wings); Twisted, *tw* (legs); Stumpy, *st* (legs); White, *wh* (eyes); Crescent, *cr* (eyes); Cut, *ct* (wings); Club, *cl* (tarsi and wings); Glass, *gl* (eyes and antennae); Indented, *in* (wings); Truncated, *td* (wings); Crepe-wings, *cw*; Strap, *sr* (wings); Gynoid, *gy* (antennae and abdominal sclerites); Extended-head, *eh* (prothorax); Honey, *ho* (body color); Pointed, *p* (wings); Aciform, *ac* (antennae); Extended-wings, *ew*.

A description of some of these mutant traits together with an account of the occurrence of the mutations has been reported by the senior author (WHITING 1932). Others are new and will be described elsewhere. The eye mutations, bar, eyeless, small and extreme-small, were found and reported by WILHELMINA F. DUNNING (1931).

TABLE 1

Percentages deviating below 50 percent with probability equal to .90 or to .99 that true value is no lower in population from which sample of a given size, n, is taken.

n	27	32	37	42	47	55	65	75	85	95	110	130
Percent (P .90)	35	37	37	38	39	40	40	41	41	42	42	43
Percent (P .99)	27	29	30	31	32	34	35	36	36	37	38	39

n	150	170	190	250	350	450	550	800	1500	2500
Percent (P .90)	44	44	44	45	46	46	47	47	48	48
Percent (P .99)	40	40	41	42	43	44	45	45	47	47

TABLE 2

Azygotic (male) frequencies of various combinations of the following genes: k, o, d, w, r, m, f, c, l, wa, br, el, sv, ma, Y, sl, ta, n, sp, v, b, tw, st, wh, cr, ct, cl, gl, in, td, cw, sr, gy, Eh, ho, p, ac, ew.

COMBINATION $a-b$	F_1 FEMALE	F_2 MALES			
		+	a	b	$a.b$
$k-o$	$k^e.o^i/o.d$	26	4	22	28
		41	7	23	27
	$k^s.o^i/w$	130	93	50	164
		83	78	66	112

TABLE 2 (*Continued*)

COMBINATION $a-b$	F_1 FEMALE	F_2 MALES			
		+	a	b	$a.b$
	$k.o^i/r.br$	47	23	34	46
		68	20	31	57
		45	19	25	23
		37	8	16	22
	$k.o^i.r/sv$	43	14	39	45
		47	16	30	32
		30	15	36	26
		31	21	15	21
	$k.o^i.r/ta$	40	10	29	26
		44	14	27	14
		31	21	15	25
		26	17	17	22
	$k^s.o^i/m$	38	69	259	664
		455	237	67	14
	$k^s.o^i/o.br$	92	104	57	116
		57	67	49	75
	$k^s.o^i/sv$	104	47	53	72
		57	51	47	72
	$k^s.o^i/ma$	43	42	71	201
		87	104		
	$k^s.o^i/Y$	22	21	8	35
		33	9	9	18
	$k^s.o^i/sl$	80	73	25	102
		34	120	15	126
	$k^s.o^i/ta$	65	47	18	101
		58	47	33	75
	$k^s.o^i/ta$	37	32	35	54
		62	35	25	52
	$k^s.o^i/o.tw$	69	42	44	69
		74	40	57	67
	$k^s.o^i/st$	9	7	6	8
		17	5	12	6
	$k^s.o^i/cl$	98	55	66	111
		26	15	15	32
	$k^s.o^i.gl/+$	15	8	13	23
		10	6	10	12
	$k^s.o^i/Eh$	171	69	111	191
		98	49	54	112

TABLE 2 (*Continued*)

COMBINATION *a—b*	F_1 FEMALE	F_2 MALES +	*a*	*b*	*a.b*
	$k^e.o^i/ac$	73	20	36	57
		58	19	31	53
k—d	k^e/d	48	32	64	34
k—w	k^s/w	180	257	149	190
k—r	*k.r/c.l*	52	5	74	13
		6	0	1	0
		13	1	4	0
		52	2	50	5
	k^s/r	205	235	186	223
	k/r	151	111	152	107
	k.r/+	715	309	683	301
k—m	k^s/m	297	733	522	251
k—f	k^s/f	497	515	379	425
k—c	*k/c*	329	38	266	15
k—l	*k/l*	133	18	119	8
k—wa	k^e/wa	188	197	166	136
k—br	*k/br*	180	146	123	72
	k^s/br	149	220	106	142
k—el	k^s/el	377	421	609	
	k^e/el	609	503	659	289
k—sv	*k/sv*	159	107	112	83
	k^s/sv	157	119	104	123
k—ma	k^s/ma	43	42	87	104
k—Y	k^s/Y	30	56	42	27
k—sl	k^s/sl	105	49	175	246
k—ta	*k/ta*	140	64	89	85
	k^s/ta	83	148	91	122
	k^e/ta	84	114	105	101
k—v	k^s/v	286	227	233	144
k—tw	k^s/tw	113	111	131	107
k—st	k^s/st	15	15	29	11
k—wh	k^s/wh	145	124	116	125
k—cr	k^e/cr	115	63	99	36
k—ct	k^e/ct	112	114	109	113
k—cl	k^e/cl	164	166	41	47
k—gl	$k^s.gl/+$	28	20	31	18
k—cw	k^e/cw	250	129	102	77
k—gy	k^e/gy	134	151	164	176
k—eh	k^e/eh	282	260	152	171
k—ac	k^e/ac	109	77	89	72
o—d	*o.d.r/*+	537	541	528	542
		976	1003		
	o.d/wa	115	92	94	101
		74	52	76	76
	o.d/el	52	53	75	70
		47	37	39	50

TABLE 2 (*Continued*)

COMBINATION *a—b*	F_1 FEMALE	F_2 MALES +	*a*	*b*	*a.b*
	$o^i.d/ma$	69	119	75	158
		62		75	
	o.d/sl.wh	64	63	71	66
		75	92	48	37
		103		128	
		139		100	
	o.d/ta	73	78	62	77
		89	77	64	51
	o.d/n	132	113	117	121
		72	72	98	74
	o.d/v	116	146	101	105
		199	182		
	$o^i.d/st$	86	102	73	67
		95	88	73	52
	o.d/+	3446	3486	3038	3004
	$o^i.d/+$	665	667	598	598
	$o.d/o^i$	30	50	48	50
o—w	*o.w/Y*	73	83	46	46
		48	47	36	49
	o.w/n	33	21	15	25
		15	12	14	13
	o.w/ho	91	79	73	77
		82	87	71	62
	o.w/+	1520	1391	1256	1327
	o/w	629	717	596	605
	$o^i/o.w$	128	130	134	130
	o^i/w	223	214	161	178
o—r	$o^i.r/m$	35	381	239	376
		217	25		
	pupae	141	10		
	o.r/wa	89	105	93	95
		91	101	66	107
	$o^i.r/el$	57	58	73	52
		29	44	22	24
	$o^i.r.Y/+$	37	32	24	19
		41	25	20	35
	o.r/sl	100	116	101	123
		99	117	109	124

TABLE 2 (*Continued*)

COMBINATION *a—b*	F_1 FEMALE	F_2 MALES			
		+	*a*	*b*	*a.b*
	$o^i.r/n$	42	29	37	49
		25	14	25	12
	o.r/sp	133	126	137	154
		90	110	82	96
	$o^i.r/o.b$	31	45	31	28
		20	25		
	o.r/+	1788	1871	1656	1820
	$o^i.r/+$	821	977	789	871
	o/r	177	166	195	202
	o^i/r	292	303	312	314
	$o.r/o^i$	76	95	127	113
o—m	*o.m/c.l*	652	71	41	335
		39	6	1	29
		62	18	3	10
		766	90	74	149
	o.m/+	390	71	25	149
	pupae			15	45
	$o^i.m/+$	169	22	18	74
	pupae				2
	o/m	180	1528	738	82
	pupae			128	23
	o^i/m	153	1341	924	108
	pupae			145	10
	$o.m/o^i$	73	588	250	31
	pupae			14	3
o—f	$o(o^i)/f$	447	450	330	315
o—c	*o.c/el*	70	67	78	79
		72	171		
o—l	$o^i.l/Y$	58	60	45	57
		56	53	48	60
	$o^i.l/+$	206	191	177	208
	o^i/l	130	134	109	141
	$o^i.l/o$	80	85	88	85
o—wa	*o.ct/wa*	70	78	56	43
		38	52	16	27
	o/wa	499	523	379	406

TABLE 2 (*Continued*)

COMBINATION	F₁ FEMALE	F₂ MALES			
a—b		+	*a*	*b*	*a.b*
o—br	$o^i.st/br$	97	96	81	86
		92	105	101	105
	o^i/br	543	542	415	401
	$o.br/o^i$	196	173	124	124
o—el	*o.el/sp*	100	99	60	
		84	94	22	
	$o^i.ct/el$	72	66	51	
		47	44	39	
	o.cw/el	61	47	23	
		49	46	85	
	o.el/+	184	193	82	
	$o(o^i)/el$	1136	1071	343	346
	o?			441	
o—sv	$o^i.st/sv$	36	38	27	13
		22	26	22	17
	o.cw/sv	234	204	63	62
		127	127	55	70
	$o^i.sv.st/ho$	26	19	28	25
		19	23	34	15
		30	20	26	13
		12	7	24	28
	o/sv	361	331	118	132
	o^i/sv	329	335	254	247
	$o^i.sv/+$	87	69	112	81
o—ma	o^i/ma	144	277	137	
	$o^i(k^s)/ma$	85	272	191	
	o/ma	167	157	96	98
o—Y	*o/Y*	238	209	205	174
	o^i/Y	205	212	187	170
	$o^i.Y/+$	208	175	145	135
o—sl	*o/sl*	336	368	331	370
	o^i/sl	153	127	154	141
o—ta	*o.sp/ta*	195	210	235	174
		80	48	61	77
	$o^i.ct/ta$	41	36	24	28
		26	29	21	35
	o.cw/ta	133	121	104	107
		59	61	42	51

TABLE 2 (*Continued*)

COMBINATION *a—b*	F$_1$ FEMALE	F$_2$ MALES +	 *a*	 *b*	 *a.b*
	o/ta	602	595	595	537
	o^i/ta	248	273	247	248
o—n	*o/n*	297	280	199	171
	o^i/n	453	484	266	179
o—sp	*o.sp/+*	430	384	151	125
	o/sp	370	379	256	300
o—v	*o/v*	217	251	199	182
o—b	$o^i.b/+$	138	133	25	33
	o^i/b	279	263	84	96
	$o.b/o^i$	62	73	20	25
o—tw	*o.tw/+*	318	324	180	157
	$o.tw/o^i$	111	113	114	124
o—st	*o/st.sr*	87	85	77	85
		91	78	99	101
	o/st	178	163	176	186
	$o^i.st/+$	351	310	326	326
	o^i/st	175	183	190	158
o—wh	*o/wh*	258	258	470	
o—ct	$o^i.ct/gl$	59	58	70	57
		79	60	67	50
	$o(o^i).ct/+$	486	453	361	367
o—cl	o^i/cl	153	177	41	47
o—gl	*o.gl/+*	128	117	109	96
	$o^i.gl/+$	23	36	16	22
	o^i/gl	129	115	146	110
o—in	*o/in*	128	137	98	122
o—td	*o/td*	134	147	133	140
o—cw	*o.cw/+*	595	543	332	325
o—sr	*o/sr*	164	170	190	179
o—gy	*o/gy*	138	136	118	159
o—Eh	o^i/eh	240	302	147	176
o—ho	*o/ho*	164	156	153	149
	o^i/ho	107	82	92	68
o—ac	o^i/ac	93	93	77	84
o—ew	o^i/ew	95	84	68	69
d—w	*d/w*	736	813	161	133
	d?			1028	
d—r	*d.r/+*	1078	1070	1979	
	d/r	335	260	42	584
d—f	*d.f/+*	46	41	29	26
	d/f	205	147	106	109
d—c	*d/c.l*	37	85	10	5
		6	4	84	26

TABLE 2 (*Continued*)

COMBINATION	F₁ FEMALE		F₂ MALES		
a−b		+	*a*	*b*	*a.b*
	d.n/c.l	12	4	0	2
		31	118	11	6
		31	18	290	87
		0	0	2	0
	d.c/cr	296	61	110	237
		279	51	128	235
	d.c/ho	17	16	27	98
		83	73	9	41
	d.c/ac	103	35	53	77
		105	12	65	74
	d.c/+	691	208	273	643
	d/c	172	303	505	151
d−l	*d/l*	107	220	413	135
d−wa	*d/wa*	207	195	126	152
d−el	*d/el*	432	482	214	216
d−sv	*d/sv*	133	78	142	81
d−ma	*d/ma*	69	75	62	75
d−Y	*d/Y*	100	107	116	111
d−sl	*d/sl*	230	265	306	185
d−ta	*d/ta*	151	139	166	115
d−n	*d.n/+*	333	111	50	124
	d/n	245	238	144	172
d−v	*d/v*	262	206	381	
d−st	*d/st*	188	140	183	125
d−wh	*d/wh*	294	222	242	228
d−cr	*d/cr*	406	298	407	286
d−in	*d/in*	93	97	70	101
d−td	*d/td*	67	78	60	69
d−ho	*d/ho*	44	114	92	114
d−ac	*d/ac*	156	112	170	86
w−r	*w/r*	261	168	260	204
w−f	*w/f*	616	550	375	366
w−c	*w/c.l*	69	98	21	10
		14	14	82	57
	w/c	139	161	149	116
w−l	*w/l*	127	161	138	105
w−el	*w/el*	201	151	81	201
w−sv	*w/sv*	157	76	155	47
w−Y	*w/Y*	155	92	95	85
w−ta	*w/ta*	180	171	185	143
w−n	*w/n*	54	40	27	27
w−ho	*w/ho*	170	150	169	133
r−m	*r/m*	416	615	242	

TABLE 2 (*Continued*)

COMBINATION	F$_1$ FEMALE	F$_2$ MALES			
a—b		+	*a*	*b*	*a.b*
r—f	*r.f/+*	859	750	764	823
	r/f	743	930	699	472
r—c	*r/c.l*	736	611	92	82
		87	82	636	660
	r.c/n	18	9	103	49
		51	21	10	6
	r/c	1065	941	966	970
	r.c/+	69	30	113	55
r—l	*r/l*	1017	901	912	914
r—wa	*r/wa*	194	188	192	172
r—br	*r.br/sv*	76	81	48	45
		56	38	46	35
	r.br/+	282	295	206	163
	r/br	150	176	112	83
r—el	*r/el*	947	835	575	536
r—sv	*r/sv*	265	251	209	161
r—Y	*r.Y/+*	69	43	66	55
	r/Y	93	75	79	71
r—sl	*r/sl*	313	322	290	318
r—ta	*r/ta*	149	133	119	97
r—n	*r/n*	749	713	405	285
r—sp	*r/sp*	259	291	200	178
r—v	*r/v*	140	92	51	
r—b	*r.b/+*	47	46	9	
	r/b	516	397	38	19
	r?			45	
r—st	*r/st*	49	57	33	35
r—wh	*r/wh*	109	94	122	114
r—ct	*r/ct*	233	222	183	179
r—gl	*r/gl*	111	695	763	108
r—in	*r/in*	141	157	111	91
r—td	*r/td*	93	152	145	
r—gy	*r/gy*	130	319	151	331
f—c	*f/c.l*	86	52	13	6
		8	5	106	41
	f/c	228	146	244	153
f—l	*f/l*	473	378	495	212
f—el	*f/el*	264	224	220	183
f—sv	*f/sv*	72	86	79	65
f—v	*f/v*	203	122	111	50
f—b	*f/b*	14	3	4	7
f—wh	*f/wh*	139	160	150	144

TABLE 2 (*Continued*)

COMBINATION $a-b$	F_1 FEMALE	F_2 MALES			
		+	a	b	$a.b$
$f-ct$	f/ct	112	57	116	33
$f-gl$	f/gl	140	139	196	72
$f-in$	f/in	294	135	142	103
$f-td$	f/td	86	74	73	4
$f-cw$	f/cw	130	136	131	128
$c-l$	$c.l/br$	184	19	12	182
		181	8	19	152
	$c.l/Y$	67	6	15	63
		42	3	3	23
	$c.l/ta$	141	7	19	138
		105	16	10	127
	$c.l.n/+$	742	160	1	14
		8	1	41	320
	$c.l/+$	5472	658	588	5131
	c/l	36	279	303	38
$c-wa$	c/wa	240	237	152	144
$c-br$	c/br	196	201	208	160
$c-el$	c/el	137	157	148	
$c-sv$	c/sv	148	128	174	141
$c-ma$	c/ma	200	399	173	
$c-Y$	c/Y	111	93	61	36
$c-sl$	c/sl	111	99	111	91
$c-ta$	c/ta	338	574	274	496
$c-n$	$c.n/+$	743	174	49	321
	c/n	92	531	227	35
$c-v$	c/v	157	185	145	128
$c-st$	c/st	128	141	115	112
$c-wh$	c/wh	337	359	615	
$c-cr$	c/cr	357	347	330	363
$c-cl$	c/cl	60	55	25	22
$c-gl$	c/gl	165	169	160	159
$c-in$	c/in	147	144	137	136
$c-cw$	c/cw	115	172	129	98
$c-gy$	c/gy	154	183	171	200
$c-ho$	c/ho	33	125	156	50
$c-ac$	c/ac	138	130	117	139
$l-br$	l/br	203	194	197	171
$l-el$	l/el	679	646	461	331
$l-Y$	l/Y	226	234	191	168
$l-ta$	l/ta	148	157	121	137
$l-n$	$l.n/+$	902	15	9	361
	l/n	19	449	182	2
$l-b$	l/b	117	116	11	0
$wa-br$	$wa.sl/br$	67	209	204	39
		86	173	220	47
	wa/br	269	725	744	167
$wa-el$	wa/el	169	162	110	127

TABLE 2 (*Continued*)

COMBINATION	F$_1$ FEMALE	F$_2$ MALES			
a—b		+	*a*	*b*	*a.b*
wa—sv	*wa.sl/sv*	83	107	113	80
		89	102	101	72
	wa/sv	289	341	338	260
wa—sl	*wa.sl/+*	467	435	496	394
wa—ta	*wa/ta*	83	98	77	41
wa—st	*wa/st.wh*	270	75	13	5
		24	6	261	79
	wa/st	294	81	274	84
wa—wh	*wa/wh*	645	352	628	343
wa—ct	*wa/ct*	148	99	90	43
wa—Eh	*wa/eh*	271	278	215	169
br—el	*br.gy/el*	31	34	585	311
		574	549	33	21
	br/el	787	797	828	403
br—sv	*br/sv*	296	194	205	175
br—sl	*br/sl*	276	243	259	267
br—ta	*br/ta*	161	124	147	150
br—b	*br/b*	196	170	47	17
br—st	*br/st*	193	167	197	206
br—gy	*br.gy/+*	616	345	607	570
el—sv	*el/sv*	163	145	181	99
el—Y	*el/Y*	412	337	235	84
el—sl	*el/sl*	305	144	159	120
el—ta	*el/ta*	545	481	473	425
el—n	*el/n*	145	99	71	86
el—sp	*el/sp*	199	60	178	22
el—tw	*el/tw*	423	296	263	152
el—cr	*el/cr*	216	181	216	
el—ct	*el/ct*	138	51	91	39
el—cw	*el/cw*	108	23	95	85
el—gy	*el/gy*	112	1157	1484	80
sv—ma	*sv/ma*	90	150	112	98
sv—sl	*sv/sl*	190	193	191	173
sv—ta	*sv/ta*	183	197	168	187
sv—n	*sv/n*	98	83	56	51
sv—v	*sv/v*	106	80	108	
sv—b	*sv/b*	246	194	60	74
sv—tw	*sv/tw*	95	138	64	62
sv—st	*sv.st/+*	95	61	92	101
	sv/st	260	167	160	156
sv—wh	*sv.wh/cr*	125	20	81	21
		93	40	71	15
	sv.wh/td	36	126	46	119
		136		121	27

TABLE 2 (*Continued*)

COMBINATION *a—b*	F_1 FEMALE	F_2 MALES +	*a*	*b*	*a.b*
	sv.wh/eh	116	34	87	52
		70	0	50	26
	sv.wh/+	567	343	467	350
	sv/wh	292	243	276	271
sv—cr	*sv/cr*	206	41	164	55
sv—cl	*sv/cl*	280	278	26	62
sv—gl	*sv/gl*	140	76	153	73
sv—td	*sv.(wh)/td.(wh)*	62	326	322	34
	sv.wh/td	36	126	136	
		46	119	121	27
sv—cw	*sv/cw*	438	125	354	125
sv—Eh	*sv/eh*	203	86	120	26
sv—ho	*sv/ho*	87	102	69	91
sv—ac	*sv/ac*	129	82	106	59
ma—sl	*ma.sl/+*	248	154	202	147
ma—ta	*ma/ta*	117	86	94	75
ma—wh	*ma/wh*	258	174	438	
ma—td	*ma/td*	85	194	178	54
Y—sl	*Y/sl*	185	108	138	117
Y—b	*Y/b*	14	17	1	0
Y—wh	*Y/wh*	138	118	149	120
Y—gl	*Y/gl*	46	53	73	67
Y—in	*Y/in*	162	141	114	63
sl—ta	*sl/ta*	467	436	377	399
sl—st	*sl/st.wh*	89	62	10	7
		13	7	60	72
	sl/st	102	69	70	79
sl—wh	*sl.wh/+*	264	252	231	239
	sl/wh	99	69	73	79
sl—gl	*sl/gl*	144	138	152	132
sl—in	*sl/in*	166	160	133	89
ta—sp	*ta/sp*	883	691	281	275
ta—b	*ta/b*	52	53	34	44
ta—tw	*ta/tw*	86	151	57	81
ta—wh	*ta/wh*	176	170	135	144
ta—ct	*ta/ct*	77	52	55	56
ta—gl	*ta/gl*	191	152	128	131
ta—cw	*ta/cw*	254	211	120	93
v—tw	*v/tw*	218	176	160	68
v—wh	*v/wh*	179	159	191	130
v—gy	*v/gy*	160	95	152	86
v—ho	*v/ho*	34	10	33	14
v—p	*v/p*	37	57	33	
st—wh	*st.wh/+*	496	45	50	472
	st/wh	60	406	400	55
st—sr	*st.sr/+*	172	162	169	200
st—ho	*st/ho*	98	91	89	71

TABLE 2 (*Continued*)

COMBINATION	F_1 FEMALE	F_2 MALES			
a–b		+	*a*	*b*	*a.b*
wh–cr	*wh/cr*	145	102	133	86
wh–ct	*wh/ct*	127	142	152	143
wh–gl	*wh/gl*	163	147	127	142
wh–td	*wh/td*	380	393	326	345
wh–gy	*wh/gy*	317	302	308	327
wh–Eh	*wh/eh*	150	139	70	76
cr–gy	*cr/gy*	85	85	75	76
cr–ho	*cr/ho*	92	99	80	116
ct–gl	*ct/gl*	117	127	139	117
cl–ho	*cl/ho*	131	123	111	119
gy–ho	*gy/ho*	126	127	120	113
gy–ac	*gy/ac*	36	215	174	31
Eh–ho	*eh/ho*	177	112	173	91

KEY TO TABLE 2

The list of genes given at the top of table 2 is arranged for convenient reference to the combinations presented in the left hand column. Combinations of kidney, *k*, made with each successive gene are presented followed by combinations of orange, *o*, made with each successive gene, et cetera. Thus if it is desired to know what tests if any have been made between any two genes, locate the group of combinations with the gene given first in the reference list. Combinations with succeeding genes will be found here in order.

The second column gives the mutant factors for which the F_1 females are heterozygous with / dividing contributions from the different parents.

The four succeeding columns (3 to 6) show the numbers of F_1 males arranged as wild-type, +(column 3), mutant type *a* (column 4), mutant type *b* (column 5), and the double mutant type *a.b* (column 6). In any given case factor *a* and factor *b* may be determined from column 1 (*a–b*). When a third mutant factor, *c*, appears in the formula of the F_1 female (column 2), males showing the trait are placed in their proper column (as regards *a* and *b*) in the next row below. When a fourth factor, *d*, appears in the F_1 formula, a third line is used, with a fourth line for the males showing both *c* and *d*. While *a* and *b* must be determined from column 1, *c* and *d* must be determined from the F_1 formula, in which *c* is placed so that it precedes *d*. Thus under combinations of *o* with *d* (*o–d*), we find F_1 female *o.d/sl.wh*. F_2 males are arranged as follows:

+	64,	*o*	63,	*d*	71,	*o.d*	66.
sl	75,	*o.sl*	92.	*d.sl*	48,	*o.d.sl*	37.
wh	103,			*d.wh*	128,		
sl.wh	139,			*d.sl.wh*	100,		

A vertical summary should give +, *o*, *d*, *o.d* (+, *a*, *b*, *a.b*) respectively, while a horizontal summary should give +, *sl*, *wh*, *sl.wh* (+, *c*, *d*, *c.d*) respectively, but in the present instance it is obvious that white-eyed wasps are indeterminable for orange. The white groups are therefore double and vacant spaces indicate masking effects. In the case of offspring from orange-ivory compound (o/o^i) females, orange males are recorded in + column.

Progenies of females heterozygous for more than two factors are in most cases included also under each possible combination of two. Thus the 636 males from $o^i.d/st$ are included with the 2528 from $o^i.d/+$, the 706 from o^i/st, and are repeated for d/st.

BIOLOGICAL AND STATISTICAL SIGNIFICANCE OF DATA

Let *AB*, *aB*, *Ab*, and *ab* represent the frequencies of the four phenotypes of offspring expected from heterozygous females *a.b*/+ (coupling test) or *a*/*b* (repulsion test). It may be supposed that wild-type, *AB*, is the most viable, but somatic overlapping may increase the relative number of *AB* and of *aB* phenotypes at the expense of *Ab* and *ab* respectively, or the reverse may occur. It may also happen that *AB* will be increased at the expense of *aB*, while *Ab* is not increased at the expense of *ab*. This may be due to the influence of factor *B versus b* or to a modifier of trait *A versus a* linked with factor *B* or *b*. A factor preventing overlapping acts as a "differentiator" (BRIDGES 1919, p. 268).

AB may excede *aB* due to differential viability in which case we might expect a comparable excess of *Ab* over *ab*. However, the ratio *ab*/*AB* cannot be predicted from $\frac{aB}{AB}\times\frac{Ab}{AB}$ since in some cases the double mutant type fails to be reduced proportionally to the single while in others the double may be highly lethal although one or both of the singles may show viability equal to that of wild-type under the conditions of culturing.

Linkage of one of the genes (*A* or *a* for example) with a lethal or semilethal may cause an excess or a deficiency of a mutant type of normal viability. Thus *aB* may surpass *AB* or the reverse. In this case we may expect a corresponding shift between *ab* and *Ab*, but such may not be obvious because of viability differences or somatic overlaps between the various combinations of *A* and *a* with *B* and *b*, or because of linkage of a second lethal or semilethal with *B* or *b*. While lethals are invisible in the material of table 2, semilethals may be either visible or invisible. Complete lethals, dying as pupae, which may be identified as to a second trait (eye color for example), have been shown in Habrobracon.

In case of linkage between *a* and *b*, $\frac{AB}{AB+aB}$ might be expected to equal $\frac{ab}{Ab+ab}$ were it not for one or more of the three disturbing factors,—somatic overlapping, non-proportionate differential viability, and linked lethals or semilethals.

It is obvious that what has been said as to the relations of *a* and *b* may also apply to relations of either *a* or *b* with a third factor *c*, or a fourth factor, *d*. It is sometimes necessary to make comparisons of pairs of frequencies as regards *a* and *b*, separately for groups *CD*, *cD*, or *Cd* since either *c* or *d* may affect phenotypic ratios of *a* or *b*.

Table 1 has been prepared in order that the reader may readily estimate the statistical significance of various pairs of frequencies occurring in the summaries of table 2. By dividing the lower frequency by the sum (n) a percentage is obtained deviating more or less below 50 percent. ($P=.90$) are those percentages at or below which 10 percent of the samples of a given size (n) will be expected to fall, while ($P=.99$) are those percentages at or below which 1 percent of the samples of corresponding size will fall. The reader may then be 90 percent sure that there is a significant difference from equality between the two frequencies if the percentage is as low as or lower than ($P=.90$) and he may be 99 percent sure if it is as low as or lower than ($P=.99$). Whether these statistically significant deviations indicate somatic overlapping, differential viability, linked lethals, linkage of the two genes under consideration, or some combination of these conditions must be judged in the individual case from the nature and variability of the traits and comparison of the different percentages with each other and with the relation of the factors in the F_1 parent.

Whether there is linkage and what may be the best estimate of the percentage of crossovers cannot necessarily be determined from the ratio $\frac{AB+ab}{AB+aB+Ab+ab}$.

MULLER (1916) has shown convenient methods of determining gametic ratio, recombinations/straights, which compensate for viability differences. If viability be affected proportionally by two pairs of allelomorphs regardless of their combination and if the numbers of the genotypes be *AB*, *aB*, *Ab*, and *ab* respectively, the gametic ratio, *r/s*, equals $\sqrt{\frac{AB\times ab}{aB\times Ab}}$ for the repulsion test and $\sqrt{\frac{aB\times Ab}{AB\times ab}}$ for the coupling test. If, however, the factors differ in their effects on viability according to their various combinations with each other and if genotypes from the repulsion cross be AB_1, aB_1, Ab_1, ab_1 and from the coupling cross AB_2, aB_2, Ab_2, ab_2, then gametic ratio should be equal to any one of the four expressions:

$$\sqrt{\frac{AB_1 \times Ab_2}{AB_2 \times Ab_1}},\ \sqrt{\frac{AB_1 \times aB_2}{AB_2 \times aB_1}},\ \sqrt{\frac{ab_1 \times Ab_2}{ab_2 \times Ab_1}},\ \sqrt{\frac{ab_1 \times aB_2}{ab_2 \times aB_1}}.$$

This second method, the method of reverse crosses, does not compensate for linked lethals, but comparison may be made of the six ratios obtainable (two by first method in case reverse crosses are made and four by second) and in case of differing results presence of linked lethals determined.

MULLER'S method does not compensate for somatic overlaps or for difficulty in determination of types, in which case phenotypic ratio involves not only deficiency of certain types below gametic ratio but also excess of other types. If viability difference be absent between A and a and if overlapping be from a to A in the same proportion whether B or b be present, then the phenotypic distribution AB, aB, Ab, ab may be corrected as follows. Decrease AB by subtracting $\frac{AB+Ab-aB-ab}{2(AB+Ab+aB+ab)} \times aB$ and increase aB by the same amount. Also decrease Ab by subtracting $\frac{AB+Ab-aB-ab}{2(AB+Ab+aB+ab)} \times ab$ and increase ab by the same amount. In other words each of the two numerically deficient groups should be increased at the expense of its corresponding excess group by a proportion of itself equal to one-half the difference between the sum of the deficient groups and the sum of the excess groups to the total.

The gametic ratio calculated after this correction is made will in case of linkage always give fewer recombination types than the original data. If overlapping of one difference be always in the same direction and unaffected by the other difference or correlated differences, the gametic ratio obtained should be correct. Even if overlapping be in both directions (from A to a as well as from a to A), recombinations after correction should be fewer than before. True crossover value will not be greater than that indicated by ratio thus obtained, but it may be considerably less. The value of the method lies in the fact that it gives a maximum which is lower than may be calculated from the frequencies directly.

DISCUSSION OF DATA

DUNNING (1931) reports a female-sterile mutant type with bar eyes. Her data indicate independent segregation with orange, defective, reduced and eyeless. With reference to the last, F_2 from eyeless females by bar males consisted of wild-type 132, bar 86, eyeless 70. Bar and eyeless were both of decreased viability as compared with wild-type and it is possible that the double mutant type, bar eyeless, were lethal, as suggested by Dr. DUNNING, or that some of the F_2 eyeless phenotypes were genetically eyeless bar.

Comments will be made on data of table 2 in those cases only which involve marked deviations from expectation or other points of interest.

Kidney shows unmistakable linkage with orange. Combinations of the various allelomorphs may be summarized without regard to other genes. From $k^s.o^i/o$ females, progeny were orange 292, small orange 253, ivory 207, small ivory 327; 41.48 percent recombinations among the full-sized and 43.62 percent among the small. From $k^s.o^i/+$ (omitting those from $k^s.o^i/m$) progeny were wild-type 847, small 757, ivory 436, small ivory 1127; 33.98 percent recombinations among the full and 40.18 percent among the small. Small overlap with full and modifying factors influence counts, as may be noted among offspring from $k^s.o^i/sl$ among which semilong had unusually high proportion of small eyes. From $k.o^i/+$ females, progeny were wild-type 489, kidney 198, ivory 314, kidney ivory 359; 39.10 percent recombinations among the full and 35.55 percent among the kidney. Kidney is semilethal when wasps are reared at 30°C and hence counts show deficiency. Eyes are frequently lacking and identification by ocelli alone may account for disparity in ratio of recombinations. From $k^e.o^i/o$, progeny were orange 67, extreme-small orange 11, ivory 45, extreme-small ivory 55; 41.17 percent recombinations among the full and 16.67 percent among the extreme small. It is impossible to tell with certainty the difference between orange and ivory among the extreme-small. From $k^c.o^i/+$, progeny were wild-type 623, extreme-small 294, ivory 433, extreme-small ivory 672; 41.00 percent recombinations among the full and 30.43 percent among the extreme-small. The difference between black and ivory can be determined among extreme-small, but when eyes are lacking identification by ocelli is unreliable. The most reliable data for determining percentage of crossing over are therefore the counts of full-sized among fraternities segregating kidney or extreme-small. These total 1971 with 792 (40.18 percent) crossovers.

Summarized combinations of orange and miniature ($o-m$) show 499 (11.05 percent) crossovers among the 4515 non-miniature; 265 (11.00 percent) among the 2399 adult miniature; and 51 (13.42 percent) among the 380 lethal pupae. Crossing over may therefore be taken as approximately 11 percent. Ratio of pupae to adults is of no significance as pupae were not always counted. Ratio of adult miniature, 2399, to non-miniature, 4515 (53.13 percent), expresses relative viability of miniature.

A three point experiment planned to determine relative positions of kidney, orange and miniature yielded 1803 males from $k^s.o^i/m$ females. There were 55 percent with small eye, 56 percent ivory, and 43 percent miniature. Difficulty in identification of small makes counts unreliable. Miniature, more numerous on the average than expected, were not equally deficient in the various alternative classes. Summary of alternative classes shows straights 1119, recombinations between small and ivory 632, between small and miniature 548, and between ivory and miniature 188. From this it may be judged that miniature lies between orange and kidney, giving 136 (7.54 percent) crossovers between orange and miniature alone, 496 (27.51 percent) between miniature and kidney alone, and 52 (2.88 percent) double crossovers. From these data it may be concluded only that miniature lies at about one-fourth the distance from orange to kidney.

No linkage is indicated in combinations between kidney and any genes tested other than orange and miniature. Linkage of cantaloup and long and thermo-lethality of kidney may be noted among progeny from *k.r./c.l.* Small eye, k^s, depart widely from expectation due to overlapping and as affected by modifiers. Maroon is masked by ivory (from $k^s.o^i/ma$). Small is masked by eyeless (from k^s/el).

Mutant type offspring from k^e/el were classified into three groups,—extreme-small 503, eyeless with lobes on sides of head 659, and eyeless without the lobes 289. Significance of these types is uncertain and the problem is being further investigated.

Proportion of types from k^s/v is best explained by overlapping of small and decreased viability of vestigial.

Offspring of k^e/cr demonstrate that crescent is not allelomorphic with kidney. The k^e/cr females had eyes of approximately normal size.

Under combinations of orange with defective venation ($o-d$), it may be seen that defective is masked by reduced (from $o.d.r/+$); maroon is masked by ivory (from $o^i.d/ma$); orange is masked by white (from *o.d/sl.wh*); defective is masked by vestigial (from *o.d/v*).

Defective are of normal viability but overlap with wild-type and the trait may appear as a result of minor factors without the presence of *d*. This factor was called d_{1195} in a study of variation and modifiers (WHITING 1924).

Among progeny from *o.d/sl.wh* there appears to be linkage between *d* and *sl*, but this is a somatic effect. (See summary under $d-sl$.) Semilong contracts the end of the wing (so that vein R_4 is more likely to extend from R_3 to M_1) thus obliterating the effect of *d*. The excess of defective as compared with type is undoubtedly an effect of minor factors. Eyeless has an effect on defective somewhat similar to *sl* (from *o.d/el*) but not so extreme. (See $d-el$.)

Summaries of combinations of orange and defective ($o-d$), including much material previously published, indicate no linkage but show overlapping of defective into wild-type.

Wrinkled are somewhat less viable than wild-type, frequently failing to eclose. There were 4540 (48.6 percent) recombinations with orange among 9339.

Progeny from $o^{i}.r/m$ show linkage of ivory and miniature and low viability of miniature. Reduced were not classified as regards miniature. Lethal miniature pupae can be identified as to eye color and show linkage with orange. The lethal pupae include reduced miniature as well as miniature which accounts for the deficiency of adult reduced as compared with ivory reduced.

Among progeny from $o^{i}.r/o.b$, beaded were not classified as regards reduced. Beaded are of very low viability.

Summary of $o-r$ shows 6498 straight and 6477 recombination.

Fraternities bred from *o.m/c.l* (by C. H. BOSTIAN) show linkage of *o* and *m* (14.3 percent crossovers, vertical summary) and of *c* and *l* (7.1 percent crossovers, horizontal summary). Summarizing for miniature and long alone we have wild-type 768, miniature 405, long 936, miniature long 236, suggesting linkage (41 percent recombinations). Among the 1704 non-miniature there are 45.07 percent recombinations while among the 641 miniature there are 36.82 percent recombinations. Both of these ratios differ significantly from 50 percent (table 1), and are also widely different from each other. The case can be explained by linkage of a lethal with non-long, and by increased lethality of miniature by long. Other evidence shows that there is no linkage between miniature and long.

Among combinations $o-c$ from *o.c/el*, eyeless were classified by ocelli as black or light, the latter including orange, cantaloup, and orange cantaloup.

Among combinations $o-el$, progeny from *o.el/sp* show majority of eyeless classified as non-spread. Spread wing lightens muscle-insertion pigment on side of thorax and wasps are classified by this trait. Eyeless has a general darkening effect on body pigment which counteracts this character.

Progeny of *o.cw/el* show majority of eyeless classified as crepe-wings. Eyeless causes a certain amount of wrinkling of wings resulting in confusion.

Summaries from orange (or ivory) crossed with eyeless show F_2 males,—wild-type 1136, orange (or ivory) 1071, eyeless with black (wild-type) ocelli 343, eyeless with light ocelli 346, eyeless with ocelli unclassified 441, giving no evidence of linkage. Eyeless are of decreased viability as compared with non-eyeless sibs.

Progeny of *o.cw/sv* show deficiency of shot-veins but among crepe-wings there is apparent equality. This is because extreme shot-veins may cause sufficient wing deformity to be classified as crepe-wings. Shot-veins is a variable trait, much affected by external conditions, and overlapping with wild-type.

Maroon eye (masked by ivory or by white and classified with uncertainty in presence of orange) may segregate in equal numbers from type (from o^i/ma) or may surpass (from $o^i.k^s/ma$) or fall below (from *o/ma*, *ma.sl/+*, *ma/wh*, *ma/ta*) type. These deviations may be due to linked lethals. Maroon is linked with shot-veins and with truncated.

Under $o-n$, progeny from o^i/n show a significant deficiency of ivory among the narrow despite a slight excess of ivory among the non-narrow. Progeny from *o/n* indicate no linkage and there is no reason to suppose that ivory would itself reduce viability of narrow. Possibly there was coupled with ivory, a factor having such an effect.

Under $o-Eh$, progeny from o^i/Eh show a significant excess of ivory, similar both in extended head and in non-extended head. This is probably due to a linked lethal.

As regards $d-r$, reduced masks defective. The 626 reduced from *d/r* include 42 with R_4 complete in both wings and 584 with R_4 lacking from one or both wings. Lack of R_4 in reduced is no criterion of presence of factor *d*.

Various combinations of *d*, *c*, *l*, *n*, *cw*, and *ho* show linkage. On account of overlapping and modifiers defective show much irregularity. Low viability of narrow also disturbs ratios.

Progeny of *d.c/ho* show that honey tends decidedly to increase proportion of defective. This is due to general lightening of wing veins along with body pigment. If this group be omitted there are 1451 progeny from *d.c/+*,—wild-type 591, defective 119, cantaloup 237, and defective cantaloup 504. Cantaloup are in slight excess while defective are 42.7 percent. Apparent crossovers are increased among cantaloup, decreased among non-cantaloup by overlapping of defective into wild-type. Correction according to the method above suggested gives probable genotypes as follows: +571.4, *d* 138.6, *c* 154.1, *d.c.* 586.9. Crossover ratio by square root of product method is from uncorrected data 23.6 percent, from corrected 20.1 percent.

Defective is linked with long. From *d/l* the 875 progeny show deficiency of cantaloup (40.6 percent) and excess of long (62.6 percent) on account of overlaps and linkage with narrow. Apparent crossovers are 32.7 percent among non-long and 25.0 percent among long. The true value cannot be determined.

From *d.n/+* the 618 progeny include 38.0 percent defective and 28.2 percent narrow. Apparent crossovers are 25.0 percent among non-narrow and 28.7 percent among narrow. From *d/n* the 799 progeny include 39.5 percent narrow and 51.3 percent defective. Evidently minor factors for defective are present which explain the equality of defective phenotypes. One of these may be linked with narrow since narrow defective exceeds narrow.

From *c.l/+* the 11849 progeny include 48.9 percent cantaloup and 48.3

percent long. Crossovers are 10.5 percent. From *c/l*, the 656 progeny include 48.3 percent cantaloup and 52.0 percent long. Crossovers are 11.3 percent.

From *c.n*/+ the 1287 progeny include 38.5 percent cantaloup and 28.7 percent narrow, the deficiency of both being due to low viability of narrow. Crossovers are 16.0 percent. From *c/n* the 885 progeny include 64.0 percent cantaloup and 29.6 percent narrow, the excess of the former being due to repulsion from narrow. Crossovers are 14.0 percent.

From *c/cw* the 514 progeny include 52.5 percent cantaloup and 44.2 percent crepe-wings. Crossing over is 41.6 percent.

From *l.n*/+ the 1287 progeny include 29.2 percent long and 28.7 percent narrow, the deficiency of both being due to coupling and reduced viability of narrow. From *l/n* the 651 progeny include 69.3 percent long and 28.1 percent narrow, the excess of the former being due to repulsion from narrow. Crossovers are 2.0 percent in the coupling cross and 2.1 per cent in the repulsion cross.

The 1287 progeny from *c.l.n*/+ indicate that the order of genes is *c–l–n* with 203 (16.6 percent) crossovers between cantaloup and long, 24 (1.86 percent) between long and narrow. There are 2 (0.16 percent) doubles with 0.30 percent expected. Alternative classes show deficiency of narrow in each case.

The 612 progeny from *d.n/c.l* indicate the order as *c–l–n–d*. There are 68 (11.11 percent) crossovers between *c* and *l*, 20 (3.27 percent) between *l* and *n*, 155 (25.33 percent) between *n* and *d*. Doubles are 2 (0.33 percent) for *c–l* and *l–n* with 0.36 percent expected; 31 (5.07 percent) for *c–l* and *n–d* with 2.81 percent expected; 8 (1.31 percent) for *l–n* and *n–d* with 0.83 percent expected; and triples are 2 (0.33 percent) with 0.09 percent expected. The differences between actuality and expectation for *c–l* and *n–d* and for *l–n* and *n–d* are statistically significant suggesting negative interference. However, narrow are in each pair of alternatives numerically deficient and defective overlaps with non-defective. Hence proportion of apparent alternative pairs to each other is modified.

From *d.c/ho*, the 364 progeny include 62.6 percent defective, 48.1 percent cantaloup and 56.6 percent honey. Crossing over is 22.5 percent between honey and cantaloup. Since honey causes a large proportion to be counted as defective, defective cantaloup honey phenotypes are greatly increased at expense of cantaloup honey, and defective honey phenotypes at expense of honey. The 158 non-honey include 44 (27.8 percent) recombinations between honey and defective, 43 (27.2 percent) between cantaloup and defective, and 33 (20.8 percent) between honey and cantaloup, giving no basis for relative positions of the three genes.

Progeny of *w/el* show apparent increase of wrinkled eyeless at expense of eyeless because eyeless tends to wrinkle wings.

Combination *r–f* shows linkage. The 3196 progeny from *r.f/+* show 46.5 percent crossovers among non-fused and 48.0 percent among fused. The barely significant difference is probably due to low viability of reduced fused. Reduced are 49.2 percent of total and fused are 49.6 percent of total. The 2844 progeny from *r/f* show 44.4 percent crossovers among non-fused and 40.3 percent among fused. The difference is apparently due to low viability of reduced fused. Reduced are 49.3 percent of total while fused are only 41 percent of total. On account of lowered viability of fused in the repulsion cross, perhaps due to a linked lethal, it is preferable to derive crossover ratio from non-fused. Among 3282 non-fused from reverse crosses there are 45.5 percent crossovers.

Combination *r–gl* shows linkage. The 1677 progeny from *r/gl* show 13.1 percent crossovers.

Combination *f–gl* shows linkage. Among the 547 progeny from *f/gl*, fused were deficient (38.6 percent) while glass were normally viable (49 percent). Wild-type and fused were equal illustrating reduced viability balanced by linkage. Fused glass were very deficient as here linkage and lowered viability act in the same direction. Among the non-fused, crossovers are 41.7 percent while among the fused, crossovers are 34.1 percent. Crossovers by square root of product method are 37.7 percent.

Combination *r–b* shows great lethality of beaded.

Reduced were significantly low among progeny from *r/b* and also from *r.c/n*, *r/el* and from *r/v* (in which case vestigial masks reduced). From *r/m*, miniature were not separated among the reduced although this might have been done since reduced does not really mask miniature. Among progeny from *r/td*, reduced were in significant excess, but *td* masks reduced. From *r/gy*, reduced were over twice as frequent as non-reduced. These significant deviations of reduced in certain crosses indicate linked lethals.

F_2 males from fused usually show deficiency of fused, but those cases in which fused approximately equal non-fused indicate that dead have been removed from cocoons and counted. The group from *f/td* show reduced viability of the double recessive.

Eyeless masks cantaloup (from *c/el*) and cantaloup masks maroon (from *c/ma*).

Progeny from *c/ta* show a striking excess of cantaloup indicating one or more linked lethals. From *c/gy* there was a slight excess of cantaloup which may be similarly interpreted.

Wavy and broad show linkage. Among 1905 males from *wa/br* there are 26.5 percent crossovers among non-wavy and 18.7 percent among wavy. By square root of product method crossovers are 22.4 percent.

Progeny of *wa/sv* suggest loose linkage (44.7 percent). Progeny of *br/el* (including *br.gy/el*) show low proportion of broad eyeless. The excess of eyeless non-broad over broad non-eyeless suggests that broad overlap with non-broad among the eyeless, but reduced viability or linkage may also in part explain the proportion. Gynoid is linked with eyeless (from *br.gy/el* included also under *el*—*gy*). Crossovers are 6.6 percent. The low number of broad non-gynoid from *br.gy*/+ is thus explained.

Among progeny from *el/sp*, eyeless were unusually low. Spread cannot readily be distinguished among the eyeless which have a general darkening of body pigment obscuring the light pleural mark characteristic of spread.

Only three classes appear from *el/cr*, the double mutant type, eyeless crescent, being either masked or lethal.

The low proportion of eyeless non-crepe-wings from *el/cw* is because eyeless wrinkles wings somewhat, causing confusion. Many of those classed as eyeless crepe-wings should therefore be classed as eyeless.

Shot-veins is linked with maroon. The 450 progeny from *sv/ma* show 42.0 percent crossovers, 37.2 percent among non-maroon and 46.5 percent among maroon.

Shot-veins is variable with much overlapping and modification by environmental and genetic factors. It has the curious effect in combination with white of producing red spots in the white eyes especially in the ventral regions. Thus in fraternities here reported the double recessive, shot-veins white, is variegated. Variegation is of value in determining the presence of the factor for shot-veins even though the trait is masked in the wings, as by truncated which proves to be linked with shot-veins. Thus among progeny from *sv.wh/td* which is included both under *sv*—*wh* and *sv*—*td*, the black-eyed wasps could be separated for shot-veins only if they are non-truncated while white shot-veins truncated could be separated by eyes from white truncated. This fact was utilized in an experiment to test linkage of shot-veins and truncated in which white was present in both parental types. It may be noted that in combinations *sv*—*td* crossovers are less frequent in truncated than in non-truncated, perhaps due to decreased viability of the double recessive or to failure in counting to recognize variegated in all cases. From *sv.* (*wh*)/*td.* (*wh*) crossovers are 12.4 percent;—16 percent among non-truncated and 9.5 percent among truncated. From *sv.wh/td* crossovers are 22.2 percent among non-white non-truncated, 27.8 percent among white non-truncated and 18.2 percent among white truncated. The white in this cross average 22.7 percent crossovers.

Maroon and truncated are linked. From *ma/td* there are 26.8 percent crossovers. Shot-veins, truncated and maroon thus form a linkage group with genes arranged in that order.

Variation in ratio of shot-veins may be noted in combinations with other traits. With semilong, tapering, narrow, and possibly honey, shot-veins are approximately equal to non-shot-veins whether or not in combination with the other mutant factor. With beaded and stumpy, shot-veins deviate significantly below non-shot-veins except when in combination with the other mutant factor which may act as a differentiator. With twisted, however, there is a significant deviation in the reverse direction. Vestigial masks shot-veins. Significant deviations of shot-veins below non-shot-veins occur in combination with white, crescent, glass, crepe-wings, extended-head, and aciform. Club increases number counted as shot-veins because club itself affects venation in a somewhat similar although more restricted way and hence some club were classified as club-shot-veins.

Progeny from *sv.wh/Eh* are in unexpected proportions which apparently cannot be explained without elaborate hypotheses. No linkage of the genes under consideration is indicated. Total absence of shot-veins extended-head is far from expectation on the basis of ratio of single mutant types to wild-type.

Tapering significantly exceeds non-tapering from *ta/tw*. The difference probably due to a linked lethal, is less among twisted than among non-twisted, probably because of reduced viability of the double recessive.

Stumpy and white are linked showing 9.0 percent crossovers in the coupling cross and 12.4 percent in the repulsion.

There is an indication of possible though very loose linkage between stumpy and strap (from *st.sr/+*).

Gynoid and aciform are linked with 14.7 percent crossovers (from *gy/ac*).

AZYGOSIS IN OTHER FORMS

Segregation of gametic characters such as chromosome differences, has been shown by direct observation to result in numbers approximating the 1:1 ratio. Besides the extensive records on distribution of sex chromosomes in meiosis segregation of heteromorphic homologous chromosomes in Acridians has been reported by CAROTHERS (1926). It is well known that spermatids of many forms may be counted directly for presence or absence of the X chromosome. Measurements of sperm heads have been shown (ZELENY and FAUST 1915) to form a bimodal frequency distribution indicating presence or absence of the X.

Microspore characters in plants are for the most part parental traits having much the same relation to the nuclei within that the pericarp of grain, the color of an egg shell or the direction of coiling of the shell of Lymnaea (STURTEVANT 1923) have to their respective embryos. A few pollen characters, however, have been observed to segregate. (See SANSOME and PHILP 1932, for discussion and bibliography.) Bimodal curves of size of pollen grain have been correlated with sex chromosomes in Cannabis, Melandrium and Rumex. A great variety of pollen lethals, due to chromosome irregularities, may be directly identified by form and size of the grains, as in Datura, maize and Oenothera. Type of carbohydrate storage has also been noted in pollen of rice and maize, which by color reaction after iodine test may be separated into two distinct types in 1:1 ratio. Dimorphism of pollen determined by segregating genetic factors has been noted also in Oenothera in respect to form of starch grains.

Difference in rate of growth of pollen tubes of several plants (Oenothera, Melandrium, Rumex, Triticum, Datura, Zea) according to segregating genetic factors may be directly observed. This "certation" explains many cases of self- and cross-incompatibility.

In fungi and mosses as well as to some extent in algae, it is often possible to separate the spores of a tetrad derived from a single spore mother cell and to study the traits of the azygotes developed. This method of tetrad analysis is invaluable in the study of synapsis phenomena (GOLDSCHMIDT 1932).

ALLEN (1930) has shown segregation of traits in dioecious leafy liverworts. Bryophyte plants are azygous and haploid.

Sporophytes derived from self-fertilization of a monoecious moss plant or of a fern prothallium should be comparable with diploid azygotes in being completely homozygous. ANDERSSON-KOTTÖ (1927), working with frond characters of *Polystichum angulare*, cultivated the prothallia from each spore in isolation and found that all the sporophytes from one prothallium were alike and homozygous. Thus segregation of prothallia for two genic differences affecting fronds was noted.

Haploid sporophytes of seed plants are occasionally found resulting from development of an unfertilized egg nucleus. These azygotes are usually small, weak, and highly infertile. Self-fertilization results in completely homozygous offspring, but such are more readily obtained asexually following injury to growing points (LINDSTROM 1929). There is thus no real genetic difference to be expected between the former (zygotes) with chromosome number regulated by fertilization and the latter (azygotes) with chromosome number regulated asexually.

Haploid parthenogenesis probably occurs in frogs (PARMENTER 1926) as a result of artificial stimulation. Both haploid and diploid mitotic figures were found in impaternate tadpoles and in cleavage nuclei, in some cases in the same individual, indicating "that the diploid number is not always formed before the first cleavage." All frogs that metamorphosed (25) were diploid. Reconstitution of diploid number is indicated but "it is still possible that the diploidness of these viable individuals may have been produced before the first cleavage." If numerical regulation occurred between the second maturation division and the first cleavage (as apparently in the honey-bee) these impaternate diploid frogs would still be azygotes.

Haploid parthenogenesis occurs in a number of invertebrates. The resulting azygotes are male in Hymenoptera, Aleurodidae, Coccidae, thrips, mites, and rotifers.

Azygotic ratios have been shown in drone honey bees. NEWELL (1914) made reciprocal crosses of yellow Italian with gray Carniolan. The heterozygous yellow queens produced yellow and gray drones segregating in equal numbers. MICHAILOFF (1931) reported a recessive mutation to white eyes. Heterozygous black-eyed queens produced black-eyed and white-eyed drones in equal numbers. Males of several Hymenopterous insects are haploid at least with respect to the spermatogonia, but a diversity of opinion prevails with regard to the honey-bee. NACHTSHEIM (1913) regards the males as essentially haploid, but although the mature egg nucleus contains but eight chromosomes these become sixteen on the cleavage spindle. The embryo has 16, 32, or 64 in various tissues. Spermatogonia contain 16 chromosomes. First spermatocytes have 16 dyads, which fail to divide in the first maturation division, but fuse in pairs to form bivalents. These divide in the second maturation division so that the spermatids have 8 dyads or 16 chromatids. The male pronucleus therefore has 16 chromosomes. Since the 8 chromosomes of the mature egg nucleus divide into 16, the fusion nucleus has 32. NACHTSHEIM regards the eight chromosomes of the maturation divisions as compound, "Sammelchromosomen," and believes that 16 is the fundamental number (potential linkage groups). Since however there are eight tetrads in the first ovocyte and eight bivalent dyads (tetrads) in the second spermatocyte, the number eight may be regarded as haploid (potential linkage groups) while the 16 of the ovogonia or of the spermatogonia represent eight homologous pairs (diploid). Somatic cells of males or of females may contain two, four, or eight sets of homologues rather than fragments of the original eight chromosomes.

This process of doubling of chromosome number may be regarded as one method of regulation in order that chromatin (and genic) content may be increased. Such multiple sets have been shown by HOLT (1917) in the large larval digestive cells of Culex in which cytokinesis is inhibited during the pupal stage. Another and more usual method of regulation, probably not essentially different physiologically from numerical increase, is to enlarge size of chromosomes either in haploid or in diploid number according to the various cell sizes. Thus the haploid number is retained in many azygotes. This process of regulation is very poorly carried out in haploid sporophytes which are consequently weak and of small size.

Azygotic females probably occur in a number of invertebrates, but females of most thelytokous races and species produce their impaternate daughters by some form of diploid parthenogenesis either by suppression of second ovocyte division with or without preceding asynapsis or by refusion of second polar body with egg nucleus.

Facultative thelytoky occurs in several orthopterous insects. In the grouse locusts, Tettigidae, both genetic and cytological studies indicate that this is haploid. Virgin females heterozygous for various genes governing color patterns of pronotum and hind femora produce daughters which are in all cases (with one or two doubtful exceptions) homozygous. Cytological studies of embryonic cells show numbers varying from seven (haploid) to fourteen (diploid) chromosomes. Chromosomes of the haploid set are in transverse thickness twice the size of the diploid, so that each additional above seven in number means that one thick has been replaced by two thin. These facts seem to indicate regulation of chromatin volume followed by reconstitution of diploid number by longitudinal division. ROBERTSON (1930) and NABOURS (1930) hold that the diploidal condition is retained or restored by suppression of the second ovocyte division and that the thick chromosomes are dyads persisting into embryonic cells after diploid parthenogenesis. KING and SLIFER (1933), however, state that the diploid condition is restored in other grasshoppers after haploid parthenogenesis and that in grouse locusts, if diploid parthenogenesis resulting from suppression of second maturation division occurs, the first maturation division must be completely segregational. Since this is highly improbable and since it is unlikely that dyads should persist as such into late embryonic stages, it is better to regard these impaternate females as diploid azygotes.

SUMMARY

Azygotes are defined as impaternate organisms developed from cells (eggs or spores) which have undergone complete reduction in meiosis (haploid parthenogenesis). Azygotes may be haploid, or through chromosome splitting, diploid, tetraploid, et cetera.

The ratio in which azygotes from a heterozygous parent appear may depart widely from the gametic ratio because of masking effects, overlapping, and viability differences, but especially because of linked lethal factors to which zygotes from backcross data are relatively immune. A method for partially correcting somatic overlapping is given.

According to the work of other investigators, gametic segregation may be directly observed in chromosome differences in gametogenesis and in measurements of sperm-head size. Azygotic segregation may be noted in certain pollen-grain characters, pollen-tube growth, gametophytes from single spores in fungi, mosses and ferns, drones of honey-bees and impaternate

females of grouse locusts. Haploid parthenogenesis in seed plants and frogs is cited with numerical doubling in some cases.

The honey bee is regarded as an extreme example of this numerical increase since the haploid number, eight, occurring only in the gametocytes, is doubled in male and female pronuclei and further increased in somatic mitoses.

Azygotic ratios in Habrobracon from counts of about 186,000 males from mothers heterozygous for two or more genes are given. These involve 254 different combinations of 39 genes.

Seven linkage groups are shown with genes as follows:

1. Orange, miniature, kidney. 2. crepe-wings, honey, cantaloup, long, narrow, defective. 3. reduced, glass, fused. 4. wavy, broad. 5. eyeless, gynoid, aciform. 6. shot-veins, truncated, maroon. 7. stumpy, white. Data are not as yet sufficient to determine the exact relationships within these groups.

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