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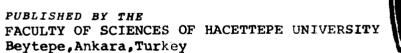
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Effects of Temperature, Light and Metabolic Inhibitors on the Mechanism of Salt Excretion in Statice species.

(Statice türlerinde tuz salınma mekanizmasına ısı, ışık ve metabolik inhibitörlerin etkisi)

## SUNA BOZCUK\*

SUMMARY: The mechanism of salt excretion by leaf discs of halophytic plants, Statice sinuata and S.latifolia under various conditions was investigated. It was found that the amount of fluid excreted increased in the light and at 20° C and decreased on metabolic inhibitors, but salt concentration of the fluid did not seem to be affected by these factors.

#### INTRODUCTION

A high concentration of salt in the soil solution has been regarded in the past as making it difficult for a plant to absorb water because of osmotic effects and this led Schimper (1891) to propose his theory of physiological dryness in respect of the salt marsh habitat. Since the days of Schimper a great deal of experimental work has been carried out by many investigators and this has contributed to a greater understanding of the halophyte-problem. For instance, there is no doubt now that halophytes by accumulating salts are able to increase their internal osmotic pressure and so can absorb water quite readily from saline media. Furthermore, they are able to regulate their internal salt concentration by elimination of large quantities of salts through special structures, the so-called salt glands, on their leaves.

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The activity of such glands is supposed to prevent accumulation of excess salts in the tissue and this glands therefore lead to increased salt tolerance. The histology of these glands for various halophytic plant species has been described by so many investigators (Ruhland, 1915; Mullan, 1931; Skelding and Winterbotham, 1939; at ultrastructural level by Ziegler and Luttge, 1966; Atkinson et al. 1967; and Thompson et al. 1969).

The most important study on the physiology of salt glands was carried out by Arisz et al. (1955) using leaf discs of <u>Limonium latifolium</u>. They showed that NaCl could be excreted against concentration gradient and that the process was temperature sensitive and required oxygen. Salt excretion in various species of mangroves has also been studied by Scholander et al. (1962) and Atkinson at al. (1967). They found that the salt excretion mechanism of <u>Aegialitis</u> (mangrove) was one of the most active salt excretion systems in plants. The excretion process selected Na in favous of K and the excretion was inhibited by uncoupling agents.

It was felt desirable to extend the work of Arisz et al.(1955) and to investigate the effect of other factors on the excretion process. Therefore, a detailed investigation was made concerning the mechanism of salt excretion by leaf discs of <u>Statice sinuata</u> and <u>S.latifolia</u> under various conditions. Here, mainly the effects of temperature, light and metabolic inhibitors on the mechanism of salt excretion will be discussed.

#### MATERIALS AND METHODS

Two halophytic plant species, Statice sinuata and S. latifolia, were grown from the seeds in a growth cabinet. When the size of the leaves was large enough, leaf discs of 1 cm. in diameter were cut from either side of the midrib. Before punching the discs, plants were transferred to saline culture prepared with Hoagland' s (1938) solution plus 0.250 M NaCl for 7 days. After the 7 davs salt pretreatment the leaf discs were punched from the leaves and quickly washed in distilled water in order to clean the discs from salt deposits on the surfaces. Then they were gently blotted with blotting paper and put on filter paper in a large petri dish containing distilled water or the solution to be tested. In each experiment every petri dish contained 40 leaf discs and 2 sets of identical petri dishes were allowed to excrete under the desired conditions, such as high and low temperatures

continuous light or darkness for 24 hours. Generally, experiments were performed in a growth cabinet, unless otherwise stated, where the desired conditions can be arranged.

After the excretion period, the liquid accumulated on the upper surface of the discs was collected and measured as described by Arisz et al. (1955). Cation content of the fluid and the leaf discs before and after the excretion period were determined by a flame photometer and chloride content was determined by a chloride meter.

#### RESULTS AND CONCLUSIONS

#### 1. The effect of temperature

The temperature effect on the excretion process was investigated by measuring excretion at 7  $^{\circ}$ C and 20  $^{\circ}$ C in the dark. In this experiment in addition to distilled water (control) a group of leaf discs was placed on NaCl solution at 7.5 x 10<sup>-3</sup> M concentration. Half of the petri dishes prepared, each containing one of the test solutions and the leaf discs, was kept in a darkened box placed in the growth cabined where the temperature was adjusted to 20  $^{\circ}$ C and the other half was kept in a similar sort of box which was placed in a cold room where the temperature was 7  $^{\circ}$ C.

The rasults of this experiment presented in TABLE I for both species show that when the discs were kept at 7 °C, the amount of fluid excreted was greatly depressed as also was amount of salt excreted, but the concentration of the fluid remained about the same in both temperatures.

The ion content of the leaf discs kept at 7 °C was reduced during the experiment but only very slightly as compared with the discs kept at 20 °C and this confirmed that very little salt excretion took place at low temperature.

It may be concluded from the results of these experiments that temperature only affected the quantity of fluid excreted but has no appreciable effect on the salt concentration. 2. The effect of light

In order to investigate the light effect on excretion of salt glands, after the usual pretreatment of the plants, discs were cut and half were kept in the dark at the same temperature. The discs were placed on distilled water (control) and NaCl  $(7,5 \times 10^{-3} \text{ M})$ concentration).

TABLE 2 presents the results of this experiment for each species studied. The table indicates that the leaf discs of both species excreted a somewhat greater quantity of fluid and of ions in the light as compared with the dark treatment, but the concentration of the fluid did not change appreciably.

The reduction in initial ion content in leaf discs during a 24 hours excretion period was greater in the light than in the dark. For example, the leaf discs of <u>S. sinuata</u> lost about 22 % of their Cl content in the light and 16 % in the dark when they were placed on water, whereas leaf discs of <u>S. latifolia</u> lost about 37 % and 28 % of their Cl content in light and in dark respectively.

From these experiments it may be concluded that light has no appreciable effect on the concentration of the fluid, but it enhanced the rate of fluid excretion.

3. The effect of metabolic inhibitors

It has been shown in a preliminary experiment that leaf discs placed on either potassium cyanide (KCN) or sodium floride (NaF) excreted less fluid, but the concentration of the liquid remained almost the same as in the controls. In order to confirm the effect of inhibitors on the process of excretion and obtain more detailed information about the relationship of excretion to metabolism, an experiment was set up with the leaf discs of plant species under investigation. The experiment was performed both in the light and in the dark. The inhibitors used in this experiment were potassium cyanide (KCN), sodium floride (NaF) and chloramphenicol (CLA) all were at 7,5 x 10<sup>-5</sup> M concentration. The results are shown in TABLE 3.

It is obvious from the results summarised in the table that all the metabolic inhibitors tested inhibited the rate of fluid excretion, as well as the salt excretion both in the light and in the dark but they had no influence on the concentration of the fluid.

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Among the inhibitors used CAM always exerted slightly less inhibition effect than NaF and KCN. Furthercore, <u>S. latifolia</u> appeared to be less sensitive to CAM and NaF but was more sensitive to KCN than <u>S. sinuata.</u>

From the present experiments it may be concluded that the metabolic inhibitors only affected the rate of fluid expression but the salt concentration of the fluid remained unaffected.

#### , DISCUSSION

Salt excretion was investigated by leaf discs of both Statice sinuata and S. latifolia plant. It was shown that the quantity of the fluid tended to increase in light and at  $20^{\circ}$  C and decrease on matebolic inhibitors, but salt concentration of the fluid was not affected by these treatment. Our results are in line with those of Arisz et al. (1955) in this respect and serve to confirm that glandular activity is a metabolic process. Additionally, if the excretion on any of the inhibitors was compared with the excretion on water (control) at each condition, it appears that the relative fluid excretion in the dark and in the light was similarly inhibited by and of the inhibitors. For example, in TABLE 3, KCN inhibited the fluid excretion of S. sinuata leaf discs about 71 % in the light while this reduction amounted to about the same percentage (% 72) in the dark. In the case of S. latifolia the rate of fluid excretion was reduced by the same inhibitor about 84 % in the light and 82 % in the dark. So it follows that inhibitors affected the excretion mechanism of salt glands equally in light and in dark. This shows that the mechanism of excretion is as sensitive in the light as in the dark, probably the same mechanism is operative in both cases.

The combined results of the experiments show that under appropriate conditions leaf discs of both species excreted considerable amounts of fluid in 24 hours when placed on distilled water and the fluid had a high salt concentration. The data indicate that excretion is an important process in previnting excessive accumulation of salt in the leaf tissue. As a rule Na<sup>+</sup> and Cl<sup>+</sup> ions are the most thoudant ions present in the excreted fluid, but K<sup>+</sup> ion concentration of the fluid is also high. Teaf discs of both species excreted very little Ca<sup>+</sup> ions and the concentration appeared to be almost the same in both species. The analytical data on salt content of the leaf material before and after the excretion period show that even when the leaf discs were placed on a dilute NaCl solution the salt content of the discs decreased considerably in spite of the fact that a continuous uptake was possible from the medium.

In general it appears from the tables (1,2 and 3) that Cl concentration of excreted fluid does not balance the total cation concentration present in the fluid. This implies that some other kind of ion species may also be present in the exudate. For example, in the case of <u>S. sinuata Cl</u> concentration appears to be somewhat greater than that the total cationic concentration determined. This suggests that Cl ions may also be excreted as other salts, e.g. Mg Cl<sub>2</sub>. On the other hand, in the exudate of <u>S. latifolia</u> leaf discs Cl concentration was found to be far more below the total cation concentration and this implies that excretion of some other anions, such as bicarbonates, nitrates and sulphates is possible. As was reported by Helder (1956) in some cases no doubt there is some calcium bicarbonate excretion which is responsible for the chalk incrustations.

According to Sutcliffe (1962)'s suggestion protein synthesis is involved in the excretion process since new membrane systems are synthesized as the cycle of vacuole formation and breakdown continues. This may explain why kinetin stimulates excretion (BOZCUK 1971) and chloramphenical inhibits it.

Further studies are required in order to give a full explanation about this unique process of salt excretion which is intimately connected with the general absorption and active transport mechanisms.

#### ÖZET

Halofitik bitki olan <u>Statice sinuata</u> ve <u>S.latifolia`nın</u> yaprak disklerinde çeşitli koşullar altında tuz salgılanmasının mekanizması incelendi. Salınan sıvı miktarının ışıkta ve 20°C de arttığı ve metabolik inhibitörlerin sıvı miktarını azalttığı, fakat sıvıdaki tuz konsantrasyonunun bu faktörlerden etkilenmediği gösterildi.

Manuscript Received in May, 1978

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# TABLE 1- Effect of TEMPERATURE on the excretion of <u>Statice sinuata</u> and <u>S.latifelia</u> leaf discs at 20 °C and 7 °C, in dark, during 24 hours.

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	Excretion Quantity of Qu On fluid excred ex			Quantity of ions excreted (ng)					ration uid (m)		löH content in leaf discs (mg/g.d. wt)			
			Na <sup>†</sup>	<b>K</b> +	Ca <sup>++</sup>	C1_		<b>x</b> +	Ca++	·01	'Na <sup>+</sup>	·K+	Ca <sup>++</sup>	61 <b>-</b>
m	Ste	tice sinuatal									+(25.37	22.78	5.94	32.41)
°0	Dist.water	144	732	353	56	1407	221	63	10	279	18,52	20.78	4.87	27.22
20	NaCl	125	696	361	58	1413	242	74	11	32.0	19.03	20.78	4.87	27.87
°°	Dist. water	28	132	70	9	249	205	ő <b>4</b>	8	251	24.10	22.15	5.70	31.11
7	NaCl	23	128	62	. 8	240	242	69	9	294	24.23	22.32	5•74	31.19
°0	S.Da	atifolia									<b>+( 8.</b> 96	37 <b>•9</b> 9	4.69	16.67)
	Dist water	92	286	273	48	579	135	76	13	177	5.91	33.81	4.03	12.00
20	NaCl	73	285	265	43	594	170	93	15	299	5.97	33.89	4.07	12.50
20 02	Dist. water NaCl	22 19	65 65	62 - 57	10 9	129 127	129 148	72 77	11 12	160 188	<b>7.</b> 35 7.44	<b>3</b> 4.,95 35.,33	4.59 4.50	14.00 14.00

<sup>+</sup>The figures in paranthesis represent the ion content in leaf discs just before the excretion period.

Φ

TABLE 2- Effect of LIGHT on the excretion of <u>Statice simulate</u> and <u>S. latifelia</u> leaf discs at 20 <sup>C</sup>C, in light and dark, during 24 hours.

	Excretion Q	<b>`</b>		ted (A	<u>g.):</u>	Ion c			<u>) (</u>	Ion content in leaf discs (mg/g.d. wt)				
			3a <sup>+</sup>	<b>x</b> +	Ca <sup>++</sup>	C1 <sup></sup>	¥a <sup>+</sup>	K+	Ca <sup>++</sup>	C1 <sup></sup>	Na <sup>+</sup>	<b>K+</b>	$Ca^{++}$	C1 <sup></sup>
뛾	S.Finuata										<b>+(28.8</b> 8	23.72	5.21	34.47
LIGHT	Dist.water	178	925	479	83	1963	226	69	12	311	20,50	20.63	4.01	26.89
ы	NaCl	151	885	447	92	1885	25	76	15	352	21.08	21.11	3.96	27.57
DARK	Dist.water	150	824	415	50	1754	239	71	8	<b>3</b> 29	21.66	21.20	4.37	2 <b>8.</b> 95
DA	NaCl	139	847	\$28	54	1775	265	79	10	360	21.95	20.87	4.32	29.30
	S.latifolia	1									<b>+</b> (9.20	32.27	3.91	18.48
텶	Dist.water	177	407	401	47	839	. 149	76	10	202	5.06	27.11	3.17~	12.64
THUR	NaCl	94	374	293	41	851	173	80	11	255	5.33	28.07	3.21	12.01
ы	Dist.water	76	271	228	33	5 <b>31</b>	155	77	11	197	5.89	29.04	3.36	13.30
DARK	NaCl	64	270	217	28	566	184	87	11	249	5.98	30.01	3.46	13.67

<sup>+</sup>The figures in paranthesis represent the ion content in leaf discs just before the excretion period.

TABLE			METABOLIC											
	latifo]	<u>11a</u>	leaf disc	s at	; 20	°c,	in	ligh	t and	dark	, dur	ing 24.	hours	۱.

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	Excretion on	Quantity of fluid excreted(mg)	Quan	tity o eted (	f ions /ug)	l 	Ion concentration of excreted fluid ( mM )				Ion content in leaf discs (mg/g.d. wt)			
			Na <sup>+</sup>	K+	Ca <sup>++</sup>	c1-	Na <sup>+</sup>	<u></u> *	Ca <sup>++</sup>	C1 <sup>-</sup>	Na	K+	Ca <sup>++</sup>	C1 <sup>-</sup>
	S. Sinu	ata									+(25.87	24.83	5.61	30.39)
	Dist.w3ter	188	1007	513	67	2223	233	70	90	33 <b>3</b>	17.85	21.60	4.09	22.79
E	CAM	90	478	232	40	1015	231	66	11	318	21 <b>.9</b> 8	23.09	4.66	26.44
LIGET	NaF	34	475	246	3 <b>7</b>	913	246	75	11	306	22.25	22.84	4.77	27.05
Ξ	KCN	55	305	163	14	602	241	76	6	308	24.06	23.83	5.22	28,87
	Dist.water	108	581	30 <b>7</b>	47	1263	234	73	11	329	19.14	22.59	4.60	25.22
	CAM	57	294	147	11	638	224	66	5	315	23 <b>.28</b>	23.34	5.33	20.35
DARK	HaF	46	269	127	18	522	254	71	10	320	23.54	2 <b>3.84</b>	5.27	2 <b>7.9</b> 6
DA	ncn	31	155	91	10	343	217	<b>. 7</b> 5	8	312	24.58	24.33	5.38	29.48
	S. latif	olia									+1 (8.87	33.12	4.28	20.44)
	Dist.water	127	485	401	46	1074	166	81	9	238	4.94	27.82	3.42	13.49
E	CAM	70	227	205	22	543	141	75	8	218	6.21	30.47	3.55	16.55
THOLI	Naf KCN	60 21	204 69	189 63	17 6	462 159	148 143	81 77	7 7	217 213	7•72 8•42	31.13 32.12	3.63 4.02	19.01 20.03
DARK	Dist.water CAM Naf KCN	75 49 38 14	283 187 144 49	249 170 130 43	30 14 12 5	626 403 303 1 <b>35</b>	164 166 165 153	85 89 88 79	10 7 8 9	235 232 225 231	5.32 6.38 8.07 8.60	29.47 30.47 31.79 32.46	3•34 3•68 3•72 4•02	14.51 16.96 19.41 20.23

The figures in paranthesis represent the ion content in leaf discs just before the excretion peried.

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GENETICS OF LONGEVITY IN DROSOPHILA

IV. The Effects of Three Autosomal Genes On The Lifespan of <u>Drosophila</u> (DROSOPHILA'da Ömür Uzunluğunun Genetiği: Üç otozomal

genin Drosophila ömrüne etkileri)

Hacer Ünlü<sup>x</sup> and A.Nihat Bozcuk<sup>x</sup>

# ABSTRACT

The specific effects of three autosomal mutant genes (<u>bw</u>:2-104.5;<u>ss</u>:3-58.5 and <u>ey</u><sup>2</sup>:4-2.0) upon the adult longevity of <u>D.melanogaster</u> have been compared at  $25^{\circ}$ C. While the mean life spans of males and females of Oregon w.t. were 59.80 ± 1.15 and 62.23 ± 1.02 days respectively, they were 28.56 ± 0.98 and 56.51 for <u>ss</u>; 53.59 ± 1.01 and 46.13 ± 1.54 for <u>ey</u><sup>2</sup> and 63.90 ± 1.47 and 48.25 ± 1.75 for <u>bw</u> again respectively. The results add further knowledge to the view that life-span is a heritable trait and changeable according to sex.

#### INTRODUCTION

It is frequently pointed out that there is some need to study the specific effects of <u>Drosophila</u> mutant genes upon longevity (Lints, 1971 and Bozcuk, 1978).

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This is therefore a continuation of a series of earlier investigations to clarify the effects of some <u>Drosophila</u> mutant genes in this respect. Earlier, the effects of various combinations of sex-linked <u>wmf</u> mutant genes on the life-span were shown in heterozygous, homozygous and hemizygous conditions (Unlü and Bozcuk, 1979 a ; 1979 b). The effects of some genotypic combinations of an autosomal (vg) and sexual (w) mutants genes on the duration of life were also studied and published (Bozcuk, 1978).

In this paper, the specific effects of three different autosomal mutant genes of separate homolog chromosomes on the longevity of adult flies will be described.

MATERIAL AND METHODS

# The flies:

The mutant strains of <u>Drosophila melanogaster</u> used in the experiments were described by Lindsley and Grell (1967) as following:i) <u>bw</u> (<u>brown</u>:2-104.5);ii)<u>ss</u> (<u>spineless</u>:3-58.5) and iii)<u>ey<sup>2</sup>(eyeless</u>:4-2.0). As a control group of <u>D.melanogaster</u>, the Oregon w.t. culture was taken. These are inbred cultures and kept in this lab. for several years.

All the flies used in the experimental populations were comtemporaneous.

The flies were kept constantly at 25°C on standard medium composed of maize flour, agar, sucrose, dried yeast and a mold inhibitor. The humidity of the experimental cabinets was 40-60 % RH. The flies were always in darkness except during transfers on to fresh medium (thrice weekly), when they were in light.

# Life tables:

Life tables were constructed on described by

# Ünlü and Bozcuk (1979a).

RESULTS

The mean life spans and other related parameters of the studied populations of Oregon w.t., bw, ss and  $ev^2$  mutants are presented in Table 1. As one can see in the table the longest lived group is the w.t. controls (61.11 days as sex-combined). The difference between the means of male and females is not signigicant. However, if it is looked at the mean longevities of mutant in comparison to that of the Oregon male controls, but extended significantly (P(0.001). The respective differences between the means of opposite sexes of brown, spineless and eyeless are all significant at various significance levels (Table 1). In fact there is almost two-fold difference between the means of spineless sexes in which females are very much longer-lived than the males. Except the males of brown, the other mutant males are shorter lived than their respective w.t. control sex.

Although the mean of <u>spineless</u> males is significantly shorter than the means of respective females, the contrast situation is hold true for the <u>brown</u> and <u>eyeless</u> mutants in which males are significantly longer-lived (P $\langle 0.001 \rangle$ ).

If the sex combined average longevities are compared from the longest to the shortest the following order will be seen. Oregon w.t. (61.11). <u>brown</u>(56.07), <u>eyeless</u> (49.86) and <u>spineless</u> (42.53).

The survival curves of all populations can be seen in figures 1,2,3 and 4. In fig.1 the typical rectangular curve (Rockstein and Higuel, 1973) of the w.t. <u>D.melanogaster</u> is noticed and there is no apparent difference between the opposite sexes. Whereas there is quite some difference between the curves of <u>brown</u> females and males, in favour of the latter (Fig. 2). In fig.3, the life curves of both sexes of <u>spineless</u> are far away from each other. The females of <u>spineless</u> live twice more than the males. So much difference between two sexes of a Drosophila genotype is not noticed up to this findings. In the 4<sup>th</sup> figure it is seen that the survival curve of males of <u>eyeless</u> mutant is more extended in comparison with the females.

# DISCUSSION

In order to add further basic knowledge to the gene-determined life-span and ageing in Drosophila three different genes of the II<sup>nd</sup>, III<sup>rd</sup> chromosomes have been studied. It has been known from the literature that the adult life-span is under the control of genom (Gonzales, 1923; Pearl et al., 1923; Clark and Gould, 1970; Lints, 1971; Gould and Clark, 1977; Bozcuk, 1978; Ünlü and Bozcuk, 1979 a and 1979b). The mutants investigated here were not studied earlier. The present findings confirm the previous reports that each gene has a sex specific effects on the longevity of the adults. For example it has been found that although Oregon w.t. flies live 61.11 days (sex-combined). brown mutant flies 56.07, eyeless 49.86 and spineless live only 41.38 days. It is specifically interesting to note that females of spineless have about twice-longer mean life span against the males (56.51 + 1.30 and 28.56 days) respectively. However in relation to sex the reverse situation is observed for eveless and brown mutants in both of which females longer-lived significantly (P(0.001). However the difference between the means of sexes of Oregon control lines is not significant. These results concerning sex difference in the

durations of life are in accordance with the findings and reports of Woodhams and Hollingswordth (1971), Lints (1971), Bozcuk (1978) and Ünlü and Bozcuk (1979b), that there is no consistent pattern with regard to sex-differences.

The mutations is thought to alter the total developmental picture as well as enzymic functions. It is quite likely that the mutant genes under study cause some modifications both in the developmental program (Medvedev, 1972) and in structure and function of enzymes, therefore they may be shortening the duration of adult life span in varying degrees.

### ÖZET

Üç otozomal genin (<u>bw</u>:2-104.5; <u>ss</u>: 3-58.5 ve <u>ev</u><sup>2</sup>:4-2.0) <u>D.melanogaster</u>'in ergin ömür uzunluğu üzerindeki özel etkileri 25<sup>o</sup>C'ta karşılaştırıldı. Oregon w.t. erkek ve dişilerinin ortalama ömürleri 59.80 <u>+</u> 1.15 ve 62.23 <u>+</u> 1.02 gün iken, <u>ss</u> için 28.56 <u>+</u> 0.98 ve 56.51 <u>+</u> 1.30, <u>ev</u><sup>2</sup> için 53.59 <u>+</u> 1.01 ve 46.13 <u>+</u> 1.54 ve <u>bw</u> için 63.90 <u>+</u> 1.47 ve 48.25 <u>+</u> 1.75 gündür. Bu bulgular, ömür uzunluğunun kalıtlanan bir özellik olduğuna ve eşeyliğine göre değişebileceği görüşüne yeni destek sağlamaktadır. Manuscript Received in May, 1979

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Group No	Genotype and	Bex	No. of flies	Mean life-span (days)	S.D.	Sex-mixed average life-span (days)	Significant differences between some groups
1	Oregon w.t.	đđ	98	59.80 <u>+</u> 1.15	11.42	61,11	1-2 <sup>c</sup>
2	Oregon w.t.	<del>2</del> 2	100	62.23 <u>+</u> 1.02	10.29		3-4 <sup>ª</sup>
3	66	రేరే	71	28.56 <u>+</u> 0.98	8 <b>.28</b>	42,53	5–6 <sup><b>a</b></sup>
4	88	₽₽	100	56 <b>.51 <u>+</u> 1.</b> 30	13.07	+24 ) )	7-8 <sup>a</sup>
							1-3 <sup>a</sup>
5	ey <sup>2</sup>	00	100	53.59 <u>+</u> 1.01	10.15	10.00	1-5 <sup>a</sup>
E	ey <sup>2</sup>	<u>9</u> 2	100	46 <b>.1</b> 3 <u>+</u> 1.54	15.43	49.86	<b>1-</b> 7 <sup>b</sup>
							2 <b>-</b> 4 <sup>a</sup>
7	bw	00	81	63.90 ± 1.47	13.23	- 4 - 4 -	2–6 <sup>8</sup>
8	bw	<b>£</b> 2	83	48.25 ± 1.75	16.02	56.07	2 <b>-</b> 8 <sup>a</sup>
		т.L.		-			6–8 <sup>°</sup>

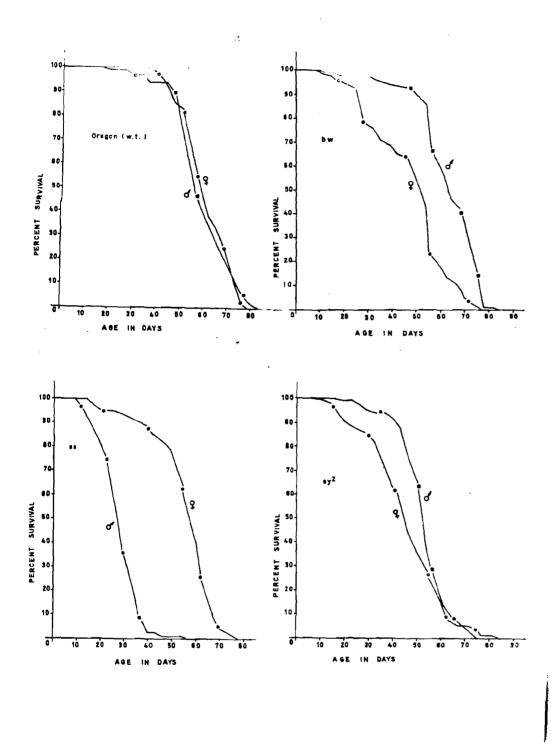
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TABLE 1: Mean life spans of the experimental genotypes in <u>D.melanogaster</u>

S.D.: Standart deviation, a and b : significant at P(0.001 and P(0.05 respectively, c: not significant.



THE CENTRAL NERVOUS SYSTEMS OF BRUCHIDAE AND CHRYSOME-LIDAE (COLEOPTERA) (Bruchidae ve Chrysomelidae (Coleoptera) Familyaları nın Merkezi sinir Sistemleri)

HALİL KASAP<sup>#</sup>

SYNOPSIS : The central nervous systems of 4 Bruchidae and 48 Chrysomelidae species are studied and compared with the published data on related species . Results of systematic importance are discussed .

INTRODUCTION : The central nervous systems of the gru ups studied consist of the brain , the subcesophageal ganglion, the thoracic and a number of abdominal ganglia. The brain, subcesophageal ganglion and thoracic ganglia are usually discrete ; the thoracic ganglia sometimes may be fused. The most important changes take place in the structure and number of the abdominal ganglia.

BLANCHARD (1846) and BRANDT (1879) studied the larval and adult nervous systems of many beetles. From their work, it is seen that the basic primitive number of the abdominal ganglia in the polyphagan larva is 8, as also found in Chrysomelids, Chrysolina hyperici Forst . (BLANCHARD , 1846) and Phaedon cochleariae F. (HAMNETT, 1944). In the adults, when some of the ganglia are fused, their morphological interpretations are difficult .In this case, it may be helpful to investigate the metamorphosis of the nervous system .Such an attempt was made by BRANDT (1879)on Coccinella 7-punctata L. (Coccinellidae ) and <u>Cetonia</u> aurata L. (Scarabaeidae ). At least being another cucujiform beetle ,C. 7-punctata is inte-resting to take a basis for the morphological interpretation of the nervous system of Cucujiformia (CROWSON, 1960), which includes the families studied here .In  $\underline{C.7}$ punctata BRANDT ( loc. cit.) figured 8 different development stage: He showed that 8 free abdominal ganglia

\* Mailing address : Çukurova Faculty of Medicine, Department of Medical Biology, Balcali-Adana-Turkey . in early instars of larva gradually changing through intermediate instars, pupa and early adult stages, finally in mature adult abdominal ganglia 1-2 became fused to the metathoracic ganglion, only the abdominal ganglion 3 remained free and the posterior ones 4-8 united together into a terminal mass of ganglia with slightly visiable divisions.

In this work, one example of the changes of the late development stage of the nervous system was found in <u>Phaedon tumidulus</u> Germ .; while in newly emerged adults (fig.19A) the abdominal ganglia 3-5 were still discrete, in mature adults only the abdominal ganglion 3 was free (fig.19B)).

By comparison it was possible to interpret the ventral nerve chains of the species . As the whole central nervous systems of the most of the species studied were figured in detail and also for the sake of conciseness, full descriptions of the central nervous system were avoided and only the number of the free abdominal ganglia was cited in the text. Therefore, it should be understood that in each species the respective abdominal ganglia anterior to free ones become fused to metathoracic ganglion and remaining posterior ones become fused together into a common mass. On the other hand ,as the connectives between the ganglia are usually double, they are reported only where otherwise .

MATERIAL AND METHODS : Where alive specimen was available, it was freshly dissected in pure water . If preserved it was at first softened in pure water for sometime, depending upon the degree of hardening and size of specimen or it was heated up in water (never boiled) to speed up softening, then dissected in usual way. The central nervous system was freed by removing the fat body and other organs . Then it was figured by free hand considering the proportions in each figure itself.

#### RESULTS

#### Family I.BRUCHIDAE

In this work the nervous systems of <u>Acanthosceli-</u> <u>des obtectus</u> (Say') (fig. 1), <u>Euspermophagus sericeus</u> (Geoffr.) (fig. 2), <u>Zabrotes subfasciatus</u> Boh. (fig. 3) and <u>Caryedon serratus</u> (Ol.) (fig.4) were studied.

In these species, meso-and metathoracic ganglia are completely fused .In <u>C.serratus</u> the abdominal ganglia 3-6 are frea and connectives between these ganglia are reduced so that the ganglia are concentrated adjoining to each other.In <u>A.obtectus</u> only the abdominal ganglion 3 and in <u>Eu. sericeus</u> 3-4 are free .Z.subfasci<u>atus</u> has no free abdominal ganglia other than a terminal mass of ganglia (fig. 3).

Family II. CHRYSOMELIDAE ( treated under following subfamilies )

1.ORSODACNINAE : The nervous system of <u>Orsodacne cerasi</u> (L.) (fig. 5) has the pro- and mesothoracic ganglia widely separated but meso- and metathoracic ganglia rather close together . The abdominal ganglia 3-5 are free and ganglion 6 is contiguous to, but well distinct from the fusion of ganglia 7-8. The connectives between the abdominal ganglia are single.

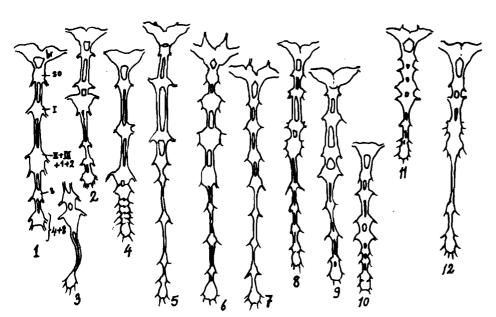
2.DONACIINAE: The nervous system of <u>Plateumaris</u> <u>sericea</u> L.was studied by BLANCHARD (1846) and those of <u>Donacia</u> vulgaris Zschach. (fig. 6) and <u>Plateumaris disoloor</u> Panz. (Tig. 7) were studied here. In these species, the abdominal ganglia 3-5 are free. The connectives between the abdominal ganglia are single only in <u>P.discolor</u>.

3. CRIOCERINAE : The nervous system of <u>Lilioceris merdigera</u> (L.) was studied by BLANCHARD (1846) and those of <u>Crioceris asparagi</u> (L.) (fig. 8) and <u>Ouleme melanopa</u> (L.) were studied in this work .In these species, the abdominal ganglia 3-5 are free. In <u>C. asparagi</u> (fig. 8) the abdominal ganglion 6 is less completely fused to the succeeding ones .

4/5. MEGALOPODINAE AND LAMPROSOMATINAE : In Zeugophora flavicollis (Marsch.) (Megalopodinae)(fig.9) the abdominal ganglia 3-5 and in <u>Oomorphus concolor</u> (Sturm.)(Lamprosomatinae)(fig. 10) the abdomnal ganglia 3-4 are free.

6. CRYPTOCEPHALINAE : The nervous system of <u>Cryptocephalus</u> (L.) was figured by BLANCHARD (1846) and of <u>Cry</u>. <u>rugicollis</u> Ol. (fi.11) was studied here. In both species only the abdominal ganglion 3 is entirely free but in <u>C. sericeus</u> abdominal ganglion 2 and in <u>C.rugicollis</u> number 4 are semidetached.

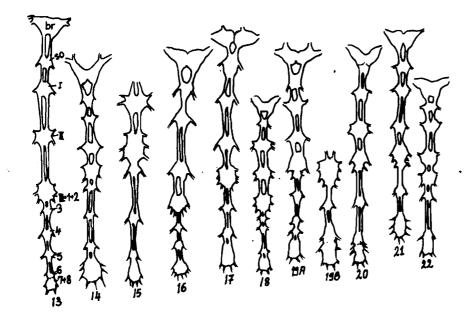
7. CLYTRINAE: The nervous systems of <u>Clytra novempuncta</u> ta Ol. (fig.12) and <u>Labidostomis taxicornis</u> F.are characterised in having meso- and metathoracic ganglia fused with a still distinguishable suture of connation. In both species the abdominal ganglion 3 is free and the ganglion 4 is still distinguishable in the mass of ganglia 4-8. The connectives between the abdominal ganglia are single in <u>C</u>. novempunctata.



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Central nervous system : (1) A.obtectus ; (2)Eu.sericeus (3) Z.subfasciatus ; (4) C.serratus ; (5) O.cerasi ; (6) D. vulgaris ; (7) P.discolor (8) Cri.asparagi ; (9) Zeu.flavicoliis ; (10) Oo.concolor ; (11) Cry .rugicollis ; (12) Cly.novempunctata .Abbreviations for all figures : br.brain ; so,suboesophageal ganglion ; I-III, thoracic ganglia ; 1-8 abdominal ganglia .

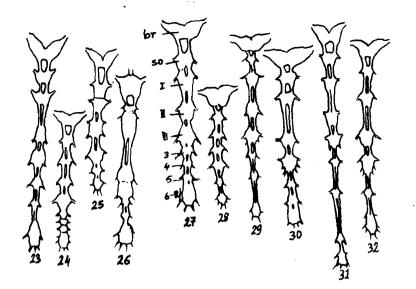
8. CHRYSOMELINAE : The nervous system of <u>Chrysolina</u> <u>hyperici</u> Forst. was studied by <u>BLANCHARD</u> (1846), that of <u>Leptinotarsa</u> <u>decemlineata</u> Say by <u>PAVLOVSKIY</u> and <u>TERAVSKIY</u> (1958), that of <u>Phaedon</u> <u>cocleariae</u> F.by <u>HAMNETT</u> (1944) and those of <u>Timarcha</u> <u>tenebricosa</u> (F.) (fig.13), <u>Entomoscelis</u> <u>adonidis</u> F. (fig.14), <u>Phyllodecta</u> <u>vulgatissima</u> (L.) (fig.15), <u>Chrysolina</u> <u>americana</u> L.(fig.16), <u>Gastroidea</u> <u>viridula</u> (DeG.)(fig.17), <u>Phytodecta</u> <u>pallidus</u> (L.) (fig.18), <u>Phaedon</u> <u>tumidulus</u> Germ. (fig.19), <u>Prasocuris</u> <u>phellandrii</u> (L.) (fig.20), <u>Hydrothassa</u> <u>marginella</u> (L.) (fig.21), <u>Placiodera</u> <u>versicolora</u> Laich. (fig.22) were <u>studied</u> here. In <u>T.tenebricosa</u>, <u>E.adonidis</u>, <u>Phyt.</u> <u>pallicus</u>, <u>Ch.polita</u> and <u>Phyl.cyanicornis</u> the obdominal ganglia <u>3-5</u> and in <u>Ch.</u> <u>americana</u>, <u>Ch.</u> <u>hyperici</u>, <u>G.viridula</u> the



(13-22) Central nervous system of Chrysomelinae, for species see the text.

abdominal ganglia 3-4 are free .In <u>Pl. versicolora</u>,<u>H.</u> <u>marginella,Pr. phellandrii</u> and <u>Ph. tumidulus</u> only the abdominal ganglion 3 is free. In <u>H.marginella</u> and <u>Pr.</u> <u>phellandrii</u> the abdominal ganglion 2 is still contiguous to anterior ones and numbers 4-5 to posterior ones and they all are still distnict .In <u>L.decemlineata</u> has no free abdominal ganglion .

9. HALTICINAE : The nervous systems of <u>Derocrepis</u> rufipes (L.) (fig.23), <u>Mantura obtusata</u> (Gyll.) (fig.24), <u>Crepi-</u> <u>dodera transversa</u> (Marsh.), <u>Chalcoides fulvicornis</u> (F.), <u>Psylloides cuprea</u> (Koch.) (fig.25), <u>Longitarsus melano-</u> <u>cephalus Deg.</u> (fig.26), <u>Aphtona coerulea</u> (Geoffr.), <u>Haltica ericeti All. Apteropeda orbiculata</u> (Marsh.) (fig.27), <u>Podogrica menetriesi</u> (Fald.) <u>Chaetocnema conci-</u> <u>nna (marsh.)</u> (fig.28), <u>Phyllotreta undulata Kutsch.and</u> <u>Sphaeroderma testaceum</u> (F.)were studied .In <u>L.melanocep-</u> <u>halus</u>, the suboesonageal ganglion with prothoracic, mesowith metathoracic ganglion are fused (fig.26).In <u>D.rufi</u>-

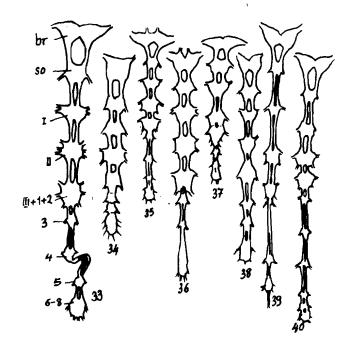


Central nervous system : (23-28) Halticinae ; (29-32) GaleruCinae, for species see the text .

pes <u>Cr.transversa</u>, <u>Ch.fulvicornis</u>, <u>Psy</u>, <u>cuprea</u>, <u>L.melanocephalus</u>, <u>Aph.coerulea</u>, <u>H.ericeti</u> and <u>Po.menetriesi</u> the abdominal ganglia 3-4 are free <u>In Ap.orbiculata</u>, <u>Ch.con-</u> <u>cinna</u> and <u>Phyl. undulata</u> only the abdominal ganglion 3 is free <u>M.obtusata</u> has no connectives between the abdominal ganglia and the ganglia 3-5 are descrete.<u>S</u>. testacuum has the abdominal ganglia concentrated in an elongate tapering mass fused to the metathoracic ganglion .

10. GALERUCINAE : The nervous system of <u>Galerucella</u> <u>birmanica</u> (Jacoby) was studied by Khatib (1946) and those of <u>Phyllobrotica</u> <u>quadrimaculata</u> (L.)(fig.31), <u>Luperus longicornis</u> (F.)(fig.29), <u>Sermylasa halensis</u> (L.)(fig.32) and <u>Lochmaea</u> <u>suturalis</u> (Thoms.) were studied here. In <u>Ph.quadrimaculata</u>, the abdominal ganglia 3-5, in <u>L.longicornis</u> numbers 3-4 are free, in other species only the abdominal ganglion 3 is free.

11. EUMOLPINAE : The nervous system of Adoxus obscurus L.was studied by BLANCHARD (1846) and those of <u>Spilopy-</u> ra sumptuosa Baly (fig.33), <u>Abirus rubripes</u> Lefev. (fig.34) <u>Scelondonta indica</u> Duviv .(fig.35), <u>Pseudopio-</u>



Central nervous system : (33) S.sumptuosa ; (34) A.rubripes ; (35) Sc.indica ; (36) Ps.andrewesi ; (37) Tricliona sp. ; (38) C.flaveola ; (39) A.balyi ; (40) H.testacea .

<u>mera andrewesi</u> Jac. (fig.36), <u>Tricliona</u> sp. (fig.37)were studied in this work. In <u>S. sumptuosa</u>, <u>Sc. indica, Chr.</u> cobaltinus and <u>A. obscurus</u> the abdominal ganglia 3-5 and in <u>Ps. andrewesi</u>, <u>Ab. rubripes</u> and <u>Tricliona</u> sp. numbers 3-4 are free .In <u>Ab. rubripes</u> all the abdominal connectives are lost and the ganglia are contiguous to each other.

12.CASSIDINAE : The nervous system of <u>Cassida equestris</u> was studied by BLANCHARD (1846), that of <u>Cassida nobilis</u> L.by BRANDT (1879) and those of <u>Cassida</u> <u>flaveola</u> Thunb. (fig 38) and of <u>Cassida rubiginosa</u> <u>Muell</u>. were studied here .Three of the species have the abdominal ganglia 3-5 free .

13. HISPINAE : The nervous system of <u>Aproida</u> <u>balyi</u> Pasc. (fig.29) and <u>Hispa</u> <u>testacea</u> L. (fig.40) were studied . While in <u>A.balyi</u>, only the abdominal ganglia 3-5 are free, in <u>H.testacea</u> numbers 3-8 are all free ; this situ-

SYSTEMATIC CONCLUSIONS :

- (a) Bruchidae distinguished from Chrysomelidae in having meso-and metathoracic ganglia fused .
- (b) In Bruchidae, Pachymerinae (<u>Caryedon</u>) can be distinguished with 4 free abdominal ganglia, Amblycerinae with either 2 free abdominal ganglia (<u>Euspermophagus</u>) or no free abdominal ganglion (<u>Zabrotes</u>) and Bruchinae (<u>Acanthoscelides</u>) with only one free abdominal ganglion.
- (c) The nervous system with 3 free abdominal ganglia is typical of eupodan subfamillies of Chrysomalidae, Orsodacninae, Donaciinae, Criocerinae and Megalopodinae.
- (d) In Camptosomata, Lamprosomatinae can be distinguished from Crytocephalinae and Clytrinae in having 2 free abdominal ganglia (numbers 3 and 4) while Clytrinae and Cryptocephalinae have only one free abdominal ganglion (number 3).Clytrinae can also be distinguished from Cryptocephalinae in having the meso and metathoracic ganglia fused.
- (e) In Chrysomelinae, most of Halticinae, Galerucinae and Eumolpinae the subcesophageal and thoracic ganglia are free with double connectives but structure of the abdominal nerve cord and the number of the free abdominal ganglia vary amongst different groups of genera in each subfamily.
- (f) In <u>Cassida</u> (Cassidinae) the basic number of the free abdominal ganglia is 2(numbers 3-4) while in Hispinae it is variable being 3 in <u>Aproida</u> ( numbers 3-5 ) but 6 in <u>Hispa</u> (numbers 3-5); in <u>Hispa</u>, probably these ganglia are secondarily separated.

ACKNOWLEDGEMENTS : My special thanks are due to Dr. R.A. Crowson of Glasgow University and also to the Zoology Department of the University for generous research facilities made available to me.

ÖZET : 4 Bruchidae ve 48 Chrysomelidae türünün merkezi sinir sistemleri çalışılarak,bulgular akraba türlerde önceden yapılmış çalışmalarla karşılaştırıldı .Sistematik önemi olan bulgular tartışıldı.

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