

Insect Virus Compilations

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manuscript of paper submitted to
JBC in 1951; rejected, then condensed
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See Correction
Table II.

Comments by Welf in red
"S.E. in pencil"

THE NUCLEIC ACIDS OF SOME INSECT VIRUSES*

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Sault Ste. Marie, Ontario.

~~With the~~ Application of recent analytical methods it has
been established that nucleic acids from various sources differ
in their contents of the purine and pyrimidine bases. These
quantitative variations presumably ^{constitute} represent one of the bases
of biological specificity in these substances.

Analyses of desoxyribonucleic acids (DNA's) from a number
of animal, plant, and microbial sources have led to the con-
clusion that DNA composition is a species character (Chargaff,
1950, 1951; Wyatt, 1951b, 1952). ^{Wyatt} While the DNA's of higher
animals are quite similar in composition, those of bacteria
and viruses exhibit more extreme variations (Chargaff, Zamen-
hof, Brawerman, and Kerin, 1950; Smith ^{and} Wyatt, 1951); among
most or all, however, certain regularities in the nucleotide
ratios are evident (Chargaff, 1951; Wyatt, 1952). Among plant
viruses, which contain ribonucleic acid (RNA), small differ-
ences in purine-pyrimidine ratios have been shown among strains
of tobacco mosaic, and these differ more markedly from other

unrelated plant viruses (Markham and Smith, 1950, 1951). It ^{therefore} thus seemed that nucleic acid analysis would aid in characterization of the ^{Insect viruses} viruses found in insects, being studied in this laboratory, and possibly provide a criterion of relationship among them.

The insect viruses comprise a [?] numerous, highly adapted, rather host-specific group (Bergold, 1947, 1948a, 1950a, 1950b; Steinhaus, 1949a, 1949b; Steinhaus, Hughes and Wasser, 1949; Smith and Wyckoff, 1951). General methods for their isolation and purification have been worked out, and their morphology extensively studied, by Bergold (1947, 1948b, 1950a). On morphological and biological grounds, they appear to form a fairly homogenous group, which may be subdivided, however, into (a) polyhedral viruses (genus Borreliina Paillot; see Steinhaus, 1949a), occurring in numbers in polyhedral inclusion bodies, and (b) capsule or granule viruses (genus Bergoldia Steinhaus), occurring singly in minute oval inclusions.

The manner of their occurrence in inclusion bodies makes it possible to prepare these viruses virtually free of host material and of cross-reaction with host antigens (Bergold & Friedrich-Freksa, 1947), a situation almost unique among animal viruses. They are relatively simple in chemical composition, having, in addition to protein, about 15% ^{per cent} of DNA (Bergold & Pister, 1948b; Smith & Wyatt, 1951), and extremely little lipid

? a
numerous
group

what about
group #3
"Smithia"
? Yes ?

or non-nucleic acid carbohydrate (Wyatt, 1950). They thus provide exceptionally favorable material for comparative studies of nucleic acid composition in related micro-organisms.

Analyses are ^{now} described of the nucleic acids of eleven species or strains of insect viruses. The results are examined for their value in characterizing the viruses, and for what light they may throw on the problems of nucleic acid composition. An analysis of the nucleic acid of one of these viruses (Smith & Wyatt, 1951), and initial results from this comparative study (Wyatt, 1952) have already been reported.

EXPERIMENTAL

Preparation of the viruses

The viruses for analysis were isolated from purified polyhedral bodies or capsules by the procedure ^{described by} of Bergold (1947, 1950a). The inclusion bodies which provided the ^{source} starting material were obtained from the cadavers of diseased insects, ^{were} and purified by sedimentation and repeated washing with water until the residue was almost white and showed very few foreign particles under the ^{light} microscope.

The standard procedure for the isolation of the viruses is as follows, details being varied only slightly to suit

the properties of the different viruses. Dry, purified polyhedra or capsules are suspended at a concentration of 5 mgm./ml. in a solution containing NaCl (or KCl) 0.05 M. and Na_2CO_3 (or K_2CO_3) 0.006 to 0.03 M.¹

¹The strength of alkali is adjusted to the solubility of the inclusion bodies of each species, as follows: Polyhedra from B. mori and C. p. eurytheme, 0.006 M.; from P. dispar, L. monacha and N. sertifer, 0.008 M.; from C. fumiferana, P. seriata, M. americanum and M. disstria, 0.03 M.; capsules from C. fumiferana and C. murinana, 0.03 M.

After 2 to 3 hours, with occasional shaking, dissolution of the inclusion body protein and liberation of the virus is complete; this can be followed with the dark-field microscope. The suspension is then centrifuged at 4000-6000 r.p.m. (Sorvall SS1 centrifuge; 1600-3600 g) for 5 minutes to remove undissolved remnants and impurities; some further virus can be recovered by suspending this residue in water and re-centrifuging. The virus is then sedimented at 11,000 r.p.m. (12,000 g) for 45 minutes and the clear supernatant liquid containing dissolved capsule or polyhedral protein is set aside. The virus is suspended in distilled water or 10^{-4} M. Na_2CO_3 of volume equal to the

see 4th line
why not express
as .0001 M

original solution, and again sedimented at 11,000 r.p.m. for 45 minutes. With some species, a brown layer consisting mainly of virus membranes (Bergold, 1952) and the undissolved surface skins of ^{of} the polyhedra lies on top of the bluish-white sediment of virus; this can often be removed by rinsing with a drop of water. The remaining sediment consists almost wholly of virus; a number of the preparations used for nucleic acid analysis have been examined in the electron microscope by Dr. G. H. Bergold. or else?

For hydrolysis, the virus is centrifuged down from a small volume of water in a 6 x 50 mm. glass culture tube, and dried. The yield is generally 2.5 - 3.5% ^{per cent} of the inclusion bodies. 60-100 mgm. of polyhedra or capsules, giving 1.5-3.5 mgm. of virus, were used for each analysis.

Hydrolysis

(70 per cent)

Perchloric acid (~~70%~~ Marshak and Vogel, 1951) ~~was~~ ^{was} used ~~been used~~ for hydrolysis, since it liberates the purine and pyrimidine bases from whole virus, along with protein degradation products which interfere little with estimation of the bases. Hydrolysis is carried out in sealed tubes in a thermostatically controlled bath. After heating,

the hydrolysate is diluted with water, and the black carbon residue is brought into suspension by grinding with a glass rod, since it tends to adsorb P. Aliquots are taken with micro-pipettes² for chromatography, P estimation, and, if desired, N estimation.

²Self-adjusting capillary type, made in the laboratory or purchased from Microchemical Specialties Ltd., Berkeley, California.

In order to obtain satisfactory emptying of the pipettes, the samples for inorganic analysis are dispensed onto small pieces of filter paper, which are dropped into flasks for incineration.

Since early experiments showed variability in the yield of thymine, some tests were carried out with purified ox spleen DNA to determine the optimal conditions. Temperatures above 100° were found to cause loss of thymine (about 15% in 1 hour at 110°). The amount of HClO₄ used is also important: with 8-15 μ l. of 70% HClO₄ per mgm. DNA, constant yields are obtained, but with greater amounts of reagent, some thymine is lost (about 2.5% with 20 μ l./mgm., and 10% with 30 μ l./mgm.). When a mixture of DNA (15%) with protein (bovine plasma albumen, 85%) was subjected to hydrolysis,

Should "C" be inserted?

the amount of HClO_4 used was less critical, and yields were unchanged over the range 10-30 $\mu\text{l. HClO}_4/\text{mgm. DNA}$. Evidently, HClO_4 has a destructive action on thymine which can be overcome by the buffering effect of either protein or sufficient nucleic acid.

From these results it seems likely that ~~even~~ ^{even} under optimal conditions with HClO_4 a small amount of thymine is lost. This would partially account for the discrepancy from the yields obtained with formic acid (Wyatt, 1951b). The method chosen, however, gave very consistent results, satisfactory for the comparison of biological materials.

The yields of bases from ox spleen DNA, with and without added protein, and the percentages of total P and N accounted for by the estimated nucleotides, are given in Table I. The change in apparent yields resulting from the presence of protein nowhere exceeds 3 per cent.

The ^{following} working procedure ^{was finally} adopted, ~~is as follows~~. To the dry virus is added 3-4 $\mu\text{l. 70\% HClO}_4$ per mgm. weight, and the tube is closed and heated to 100° for 2 hours. Sufficient water is added to make the volume of hydrolysate about 25 $\mu\text{l.}$, and the hydrolysis residue is ground into suspension. This allows two 8- $\mu\text{l.}$ portions for chromatography and two or three 2- $\mu\text{l.}$ portions for P estimation.



Estimation of the Bases

Published procedures (Markham and Smith, 1949, 1951; Wyatt, 1951b), using paper chromatography, were followed with only minor refinements in order to obtain reliable results from the least possible material. ^{Aqueous} Isopropanol (65 percent) containing ~~1.0 N.~~ HCl (2.0 N.) was used exclusively as the chromatographic solvent. Each spot was eluted in 4 ml. of 0.1 N-HCl, by standing overnight, with mechanical shaking before and after standing. It was found that ^{with} sufficient cleanliness and care ^{of} with the blanks, reliable results (difference ^s between replicates rarely exceeding 3%) could be obtained with optical densities as low as 0.1, corresponding to about 5 μ gm. of purine or pyrimidine.

Inorganic Analyses

Phosphorus (usually 1.5-4 μ gm. per sample) is estimated by the nephelometric method of Bergold and Pister (1948a), and nitrogen by a micro-Kjeldahl procedure.

RESULTS Results

All of the viruses gave rise only to the purines adenine and guanine and the pyrimidines cytosine and thymine. Neither 5-methyl-cytosine nor uracil could be detected. Some

chromatograms showed a faint spot near the position of uracil; this, however, did not have the characteristic absorption spectrum of uracil and was found to be associated with the brown "membrane" portion of a virus preparation. The cleanest white preparations gave no spot in this position, confirming the virtual or entire absence of RNA from these viruses (Bergold & Pister, 1948b; Smith & Wyatt, 1951). In one preparation of C. p. eurytheme virus, the maximum possible uracil, indicated by the absorption of an eluate from the uracil position, was equivalent to 3% of the thymine present; as this included the absorption of protein degradation products, any actual uracil must have been considerably less. The amount of DNA estimated by summing the bases corresponds to 10-15% of the various viruses, but these figures are only approximate since the procedure was not designed to refer results to dry weight.

The proportions of the several bases in the 11 viruses examined are listed in Table II. The ~~portion~~^{fraction} of total P accounted for by the estimated nucleotides averages 88%.
When compared with the corresponding figure of 100% for ox DNA analysed in the presence of protein by the same procedure, this suggests that some 10% of the virus ^P may occur

in compounds other than nucleotides.

The mean ratios of the bases from Portebetria dispar virus are in good agreement with those previously reported for the isolated nucleic acid of this virus (Smith & Wyatt, 1951).

The viruses are arranged in the table in order of increasing content of adenine. It will be noted that the thymine content of the different nucleic acids then also form an ascending series, while the values for ^{best} guanine and cytosine ^{assume a} are arranged in descending order. The ratios of adenine to thymine, and guanine to cytosine, are nearly constant for all, while the ratio of adenine plus thymine to guanine plus cytosine (AT/GC ratio) gives an index of the position of a virus in the series. These regularities are brought out in Table III. The variation in adenine/thymine and guanine/cytosine ratios within this group of organisms falls within experimental error. The corresponding ratios for ox spleen DNA are also ~~very~~ similar. These constant ratios are in accord with the regularities in the composition of DNA's which were first pointed out by Chargaff (1951) and have already been confirmed and discussed (Wyatt, 1952).

An incidental result of this balanced variation in nucleotides composition is that the mean nucleotide residue

except A & G

Not used
see Table 3

weight in each nucleic acid (i.e. the mean molecular weight of the nucleotides less 18 for water eliminated in linkage) is also constant. This figure does not fall outside the range 308.8 - 309.3 for the entire series, although the molecular weights of the nucleotides vary widely (desoxyadenylic acid 331, desoxyguanylic 347, desoxytidylic 307, thymidylic 322).

DISCUSSION Discussion

Because of their possessing surface membranes and a variety of morphological forms indicative of a life cycle, these viruses have been described as organisms (Bergold, 1950a). The term seems appropriate, and is used in this paper. Yet their possessing only one type of nucleic acid is a characteristic of fundamental importance, shared with bacteriophages (Cohen and Arbogast^S, 1950), which differentiates them sharply from bacteria ^{and} or other microorganisms with which they might be compared. Whether rickettsiae contain RNA seems not to be definitely settled (Cohen, 1950). Lack of RNA in certain viruses may be associated with their parasitic metabolism, which frees them from the need to synthesize enzyme systems. The remaining DNA has presumably genetic value.

Abence

which ones?

According to the composition of their DNA, the eleven viruses analysed have been arranged in a series with the proportions of the bases changing in a balanced manner. Their distribution in the scale, however, is not random, but shows grouping into sets with very similar or identical composition. This is most clearly shown by the AT/GC ratios in Table III, which have values of approximately 0.95 for two viruses, 1.35 for four viruses, and 1.67 for two. None of the differences in content of individual bases within the group having AT/GC ratio 1.35 (M. americanum, M. disstria, B. mori, C. p. eurythema) are significant when examined by the t test. This outcome was scarcely expected in view of the results with animal DNA's ^(Wyatt, 1951) which suggested a unique, if only slightly different, composition for each species, and those with tobacco mosaic (Markham & Smith, 1950) which indicated that even related virus strains could differ in nucleic acid composition.

There is some evidence in the results that related viruses tend to have ^{← vs →} similarly constituted nucleic acid. Thus, the two capsule viruses are close together in the scale, ^(6.2) so are the two pairs of polyhedral viruses whose hosts are members of the same ^{order} family. Such well-adapted,

is the key to the whole argument. How far any evidence of relationship among viruses, other than the DNA measurement?

*Genes DNA's related
 viruses related
 ? show
 polyhed. capsid
 All counts contained in table*

obscure - just what are you complaining?

*What does "related" mean here?
 Capsule vs. Polyhed?
 If so, note 1.67 for H. Sout.
 Tobacco is OK but not well rounded exp. in view of tip p 12*

order of families?

host-specific parasites may be expected to have evolved along with, and to some extent to parallel the phylogenetic relationships of, their hosts. No connection was obvious, however, between the morphological characteristics of the viruses (Bergold, 1952) and their nucleic acids. Their serological relations remain to be investigated.

See new page

Similarity of nucleic acid composition, on the other hand, obviously does not necessarily imply close relationship of the viruses. For example, the polyhedral virus of the sawfly N. sertifer and the capsule virus of the tortrix C. marinana are biologically as different as any pair in the series, yet have apparently identically composed DNA. The group with AT/GC ratio approximately 1.35 also includes quite unrelated ^{host} species. Yet the virtual identity of nucleic acid composition of so many species must have some significance. DNA composition in these viruses appears to change by discrete steps; yet the magnitudes of these steps are not quite sufficiently uniform to represent changes by equal numbers of nucleotides. In attempting any such explanation, it would be important to establish by further analyses whether intermediates occur. Moreover, the steps do not appear to represent mutations, since distinct viruses can have the

? Tortricid

how definite relation!

?

same DNA composition. Interpretation of these results
must probably await better knowledge of the molecular
structure of nucleic acids.

The independence of host and virus nucleic acids,
already established for bacteriophages (Smith ^{and} Wyatt,
1951) is confirmed by the analysis of a polyhedral virus
and a capsule virus from the same insect species, C.
fumiferana. These have quite distinct nucleic acids.
It would be interesting to compare the DNA's of the vari-
ous host insects. Several attempts to prepare DNA
from caterpillars led to only very small and impure
yields. Approximate analyses, however, of DNA from
B. mori and L. monacha showed that they, like other
animals, have a marked excess of adenine and thymine
over guanine and cytosine, and that of L. monacha, at
least, is quite different from its virus.

While some distinct viruses have the same nucleic
acid composition, these analyses can nevertheless take
their place among useful taxonomic characters. For
example, in a case of apparent cross-infection of L.
dispar with B. mori virus, such as was obtained by
Bergold (1943), analysis of virus DNA would establish
whether this represented true infection or provocation

? distinctly
different

- antecolant
not too
clear -
? DNA

of latent virus. The sensitivity of the method is adequate for such tests, as approximate ratios can be obtained from less than 1 mgm. of virus.

SUMMARY

Eleven viruses causing polyhedral diseases or capsule diseases of insects have been isolated and ~~sub-~~^{analysed} ~~jected to quantitative analysis~~ for the purines and pyrimidines of their nucleic acids. The viruses possess approximately 10-15% of DNA. No RNA was detected. The DNA's contain adenine, guanine, cytosine, and thymine, but no methylcytosine. The proportions of the purines and pyrimidines vary in the different species in a balanced way so that adenine/thymine and guanine/cytosine are constant ratios close to unity, but the ratio adenine + thymine / guanine + cytosine gives a characteristic index ranging from 0.71 to 1.87, by which the viruses may be arranged in a scale. Viruses possessing characteristics in common tend to fall near one another on the scale, but proximity on the scale does not necessarily imply relationship. The scale is not a continuous distribution, but contains groups of viruses having ~~identical~~ identical DNA composition, with intervals between. The

// one group comprises
poly + 1 cap

fundamental significance of these results remains uncertain at present, but it is clear that nucleic acid composition can be a useful taxonomic character for viruses.

Space >

I wish to express thanks to Dr. E.A. Steinhaus for the gift of diseased alfalfa butterfly caterpillars as a source of polyhedra, to Dr. F.T. Bird for diseased spruce ^{pine} sawfly larvae, and to Dr. G.H. Bergold for polyhedra and capsules of several species and for stimulating discussion of the work. The phosphorus and nitrogen estimations were carried out by Miss Rhona Farish.

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Table I
Yield of bases from ox spleen DNA
after hydrolysis in HClO_4 (70%, 15 $\mu\text{l.}/\text{mgm. NA}$) at 100°

Material analysed	Hydrolysis time	Moles per 100 moles total bases [*]				P accounted for, per cent	N accounted for, per cent
		Adenine	Thymine	Guanine	Cytosine		
Purified DNA	1 hr.	28.2	26.3	25.0	20.7	95	97
"	2 hr.	28.5	26.0	23.2	20.7	98	97
DNA 15%, albumen 85%	1 hr.	27.8	27.0	22.7	20.7	99	-
"	2 hr.	27.8	26.9	22.7	20.9	100	-

^{*} Allowing 1.3 for methylcytosine, not estimated in these experiments.

Table II
Purine and pyrimidine composition of DNA's of insect viruses

Inclusion body type	Host species	Host order and family	No. of analyses	Moles per 100 moles total bases				P accounted for, per cent
				Adenine	Thymine	Guanine	Cytosine	
Polyhedrus	<u>Northetria dispar</u> (L.), Gypsy moth	Lepidoptera Lymantriidae	4	21.2 ± 0.15 [#]	20.05 ± 0.18	30.5 ± 0.11	23.25 ± 0.09	92
	<u>Lymantria monacha</u> L., Nun moth	"	1	24.6	23.8	26.8	24.7	-
	<u>Choristoneura fumiferana</u> (Clem.), Spruce budworm	Tortricidae	3	24.8 ± 0.12	24.0 ± 0.09	26.7 ± 0.14	24.5 ± 0.14	86
	<u>Ptychopoda seriata</u> Schrk.,	Geometridae	2	26.7	25.7	24.4	23.2	87
	<u>Malacosoma americanum</u> (F.), Eastern tent caterpillar	Lasiocampidae	3	29.2 ± 0.22	28.0 ± 0.34	22.5 ± 0.19	20.2 ± 0.11	93
	<u>Malacosoma disstria</u> Hbn., Forest tent caterpillar	"	3	29.2 ± 0.23	28.5 ± 0.37	21.9 ± 0.19	20.3 ± 0.07	86
	<u>Bombyx mori</u> L., silkworm	Bombycidae	3	29.3 ± 0.25	28.0 ± 0.33	22.5 ± 0.05	20.2 ± 0.13	88
	<u>Golias philodice eurythema</u> Bdvl., alfalfa butterfly	Pieridae	4	29.9 ± 0.35	27.6 ± 0.08	22.4 ± 0.15	20.1 ± 0.22	90
	<u>Neodiprion sertifer</u> (Geoffr.), Pine sawfly	Hymenoptera Tenthredinidae	2	32.3	30.3	19.5	17.9	-
	Spherule	<u>Cacoecia murinana</u> Hb.	Lepidoptera Tenthredinidae*	3	32.1 ± 0.14	30.5 ± 0.44	19.7 ± 0.35	17.8 ± 0.29
<u>Choristoneura fumiferana</u> (Clem.), Spruce budworm		"	1	32.8	32.4	18.4	16.4	-

* Independent analyses performed on different preparations of virus.
Mean value and its standard error.

* = Tortricidae

Table III

Molar proportions of the bases in DNA's of insect viruses

	Virus host	$\frac{\text{Adenine}}{\text{Thymine}}$	$\frac{\text{Guanine}}{\text{Cytosine}}$	$\frac{\text{Purines}}{\text{Pyrimidines}}$	$\frac{\text{Adenine + Thymine}}{\text{Guanine + Cytosine}}$
Inclusion body type Polyhedra	<u>H. dispar</u>	1.06	1.08	1.07	0.71 364
	<u>H. monacha</u>	1.03	1.08	1.06	0.94 330
	<u>C. fumiferana</u>	1.03	1.09	1.06	0.95 260
	<u>H. seriata</u>	1.04	1.05	1.04	1.10 299
	<u>A. americanum</u>	1.04	1.11	1.07	1.34 315
	<u>A. distria</u>	1.02	1.08	1.05	1.36 324
	<u>A. mori</u>	1.04	1.11	1.07	1.34 278
	<u>C. p. eurytheme</u>	1.08	1.11	1.09	1.35 277
	<u>H. sertifer</u>	1.07	1.09	1.07	1.67 -
	Capsules	<u>A. murinana</u>	1.05	1.11	1.07
<u>C. fumiferana</u>		1.01	1.12	1.05	1.87 271
	Ox spleen DNA*	1.04	1.02	1.03	1.22

* Included for comparison; ratios are taken from the analysis of DNA in the presence of protein, and methylcytosine has been added to cytosine.

Recovery of bases from beef spleen DNA
after hydrolysis in $HClO_4$ at 100° :

Mean values from 3 experiments.

	Hydrolysis Time	Molar ratios of the bases (total = 4.00)				P recovery	N recovery
		A	T	G	C		
DNA	1 hr	1.135	1.057	0.925	0.832	94.9	97.0
DNA	2 hrs	1.144	1.046	0.931	0.830	98.4	96.6
DNA + protein	1 hr	1.119	1.086	0.913	0.831	98.6	-
DNA + protein	2 hrs.	1.120	1.081	0.911	0.839	100.4	-

Ratios in Insect Vermin NA's.

re-calculated 27-xi-51.

	A			G			C			T		
Pd 12-ii	21.2	0	0	30.6	1	1	28.1	1.5	2.25	20.2	1.5	2.25
24-iv	20.8	4	16	30.4	1	1	28.5	2.5	6.25	20.2	1.5	2.25
19-vi	21.5	3	9	30.7	2	4	28.2	0.5	0.25	19.5	5.5	30.25
15-xi	21.3	1	1	30.2	3	9	28.2	0.5	0.25	20.3	2.5	6.25
4	84.8	12	26	121.9	12	15	153.0	12	9	80.2	12	141
	21.2		2.2	30.5		1.25	28.25		0.75	20.05		3.4
		$\sqrt{= 1.5}$			$\sqrt{= 1.1}$			$\sqrt{= 0.9}$			$\sqrt{= 1.8}$	

Bm. \bar{x} 29.3 ± 0.25 22.5 ± 0.05 20.2 ± 0.13 28.0 ± 0.33

Cpe 2-iv	29.2	7	49	22.7	3	9	20.6	5	25	27.4	2	4
2-vi	29.4	5	25	22.5	1	1	20.3	2	4	27.7	1	1
3-viii	30.3	4	16	22.1	3	9	19.9	2	4	27.7	1	1
4-x	30.6	7	49	22.1	3	9	19.6	5	25	27.7	1	1
4	119.5	12	139	89.4	12	28	80.4	12	58			12
	29.9		12	22.35		2.3	20.1		4.9	27.6		0.6
		$\sqrt{= 3.5}$			$\sqrt{= 1.5}$			$\sqrt{= 2.2}$			$\sqrt{= 0.8}$	

Mq 11-viii	29.5	3	9	22.7	2	4	20.0	2	4	27.6	4	16
28-ix	28.8	4	16	22.1	4	16	20.4	2	4	28.7	7	49
14-xi	29.4	2	4	22.6	1	1	20.2	0	0	27.8	2	4
	87.7	6	29		6	21		6	8	84.1	6	69
	29.2		5	22.5		3.5	20.2		1.3	28.0		11.5
		$\sqrt{= 2.2}$			$\sqrt{= 1.9}$			$\sqrt{= 1.1}$			$\sqrt{= 3.4}$	

Md 13-xi	29.7	5	25	22.3	4	16	20.2	1	1	27.8	7	49
14-xi	29.0	2	4	21.8	1	1	20.2	1	1	29.0	5	25
15-xi	29.0	2	4	21.7	2	4	20.4	1	1	28.8	3	9
		6	33		6	21		6	3	85.6	6	83
	29.2		5.5	21.9		3.5	20.3		0.5	28.5		14
		$\sqrt{= 2.3}$			$\sqrt{= 1.9}$			$\sqrt{= 0.7}$			$\sqrt{= 3.7}$	

	A	G	C	T
Lm 18-xi	24.6	26.8	24.7	23.8
PCf 20-xi	24.6 2 4	26.8 2 4	24.4 1 1	24.2 2 4
26-xi	<u>25.0</u> 2 4	<u>26.7</u> 0 0	<u>24.4</u> 1 1	<u>24.0</u> 0 0
30-xi	<u>24.9</u> 1 <u>1</u>	<u>26.4</u> 3 <u>9</u>	<u>24.8</u> 3 <u>9</u>	<u>23.9</u> 1 <u>1</u>
	24.8 6) <u>9</u> 1.5 v = 1.2	26.7 6) <u>13</u> 2.1 v = 1.4	24.5 6) <u>11</u> 1.8 v = 1.3 1.4	24.0 6) <u>15</u> 0.85 v = 0.9
Ps 26-xi	26.7	24.3	23.2	25.7
30-xi	<u>26.6</u>	<u>24.5</u>	<u>23.2</u>	<u>25.7</u>
	26.7	24.4	23.2	25.7
CCm 1-iii	32.0 1 1	20.2 5 25	18.3 5 25	29.8 7 49
13-iv	32.0 1 1	19.8 1 1	17.8 0 0	30.4 1 1
20-iv	<u>32.4</u> 3 <u>9</u>	<u>19.0</u> 7 <u>49</u>	<u>17.3</u> 5 <u>25</u>	<u>31.3</u> 8 <u>64</u>
	6) <u>11</u>	6) <u>175</u>	6) <u>50</u>	3) <u>191.5</u> 6) <u>114</u>
	32.1 1.9 v = 1.4	19.7 12.5 v = 3.5	17.8 8.3 v = 2.9	30.5 19 v = 4.4
Ns 20-v	31.8	18.6	18.4	31.2
19-ii	<u>32.8</u>	<u>20.5</u>	<u>17.4</u>	<u>29.3</u>
	32.3	<u>19.5</u>	17.9	30.3
CCf 20-x	32.8	18.4	16.4	32.4

Summary of Virus Analyses for HA content.

Species	Date	Wt. polyphenol mg	Wt. virus mg	% yield	Volume μl	(mean units - total = 310)		
						Wt. virus in spot mg	Wt. HA in spot mg	% HA
Pd	12-ii	100	?					
	24-iv	105	3.1	2.95	16 (16.3)	1.10	0.153	13.9
	19-vi	80	1.8	2.25	14.2 (15.6)	1.35	0.246	17.5
	15-xi	60	1.2	2.0	25 (25.9)	0.38	0.55	14.5
					2.40			
Bm	11-iv	-	2.0	-	16 (17.5)	0.74	0.070	9.5
	2-vi	100	3.5	3.5	28.6 (31.2)	1.33	0.110	8.4
	3-viii	100	3.8	3.8	25 (27.8)	1.14	0.085	7.5
				3.7				
Cpe	2-iv	100	2.6	2.6	16 (17.9)	0.95	0.104	11.0
	2-vi	100	3.2	3.2	28.6 (31.0)	1.23	0.147	11.9
	3-viii	100	4.3	4.3	25 (28.2)	1.27	0.121	9.5
	4-x	200	5.9	2.95	50 (54.3)	1.80	0.190	10.5
				3.26				
Cm	13-iv	174	4.8	2.8	28 (31.5)	1.06	0.183	17.2
	20-iv	109	3.0	2.8	16 (18.1)	1.38	0.133	9.7
				2.8				
Ns	20-v	80	1.9	2.4	16 (17.4)	0.84	0.063	7.6
	19-vi	80	1.4	1.4	14.2 (15.0)	0.88	0.089	10.2
				1.9				

Species	Date	WT. polyphenol mg.	WT. virus mg.	sp. field %	Volume ↑ μl	WT. virus in sp. field mg.	WT. HA in sp. field. mg.	σ ₀ NA.
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Ma	11-VIII	100	3.6	3.6	25 (27.6)	1.08	0.124	12.5
	29-ix	80	3.6	4.5	25 (27.6)	1.08	0.136	12.4
	14-xi	60	1.8	<u>3.0</u>	26 (27.3)	0.55	0.65	11.8
				3.7				

Ma	13-xi	60	1.6	2.7	31 (32.2)	0.41	0.041	10.0
	14-xi	60	1.4	2.4	26 (27.0)	0.43	0.059	13.7
	15-xi	80	1.8	<u>2.25</u>	26 (27.3)	0.55	0.065	11.8
				2.44				

Lm	19-xi	25	0.7	2.8	8 (8.6)	0.61	0.069	11.3
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PCF	20-xi	60	1.4	2.3	25 (26.0)	0.45	0.041	9.0
	26-xi	80	1.5	1.9	26 (27.1)	0.46	0.059	12.8

CCF	20-xi	75	2.4	3.5	26 (27.8)	0.72	0.072	10.0
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P ₂	26-xi	80	1.9	2.4	26 (27.4)	0.58	0.072	12.3
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Table I
 yield of bases from ox spleen DNA
 after hydrolysis in $HClO_4$ (70%, 15 μ l./mg. NA) at 100°

Material analyzed	Hydrolysis time	Moles per 100 moles total bases*				P ac- counted for per cent	N ac- counted for per cent
		Adenine	Thymine	Guanine	Cytosine		
Purified DNA	1 hr.	28.2	26.3	23.0	20.7	95	97
Purified DNA	2 hr.	28.5	26.0	23.2	20.7	98	97
DNA ¹⁵ %, albumin ⁸⁵ %	1 hr.	27.8	27.0	22.7	20.7	99	-
"	2 hr.	27.8	26.9	22.7	20.9	100	-

* Allowing 1.3 for methylcytosine, not estimated in these experiments.

Table III

Molar proportions of the bases in DNA's of insect viruses.

Inclusion bodies	Virus host	$\frac{\text{Adenine}}{\text{Thymine}}$	$\frac{\text{Guanine}}{\text{Cytosine}}$	$\frac{\text{Pyrimidines}}{\text{Pyrimidines}}$	$\frac{\text{Adenine} + \text{Thymine}}{\text{Guanine} + \text{Cytosine}}$
Polyhedra	<i>P. diapa</i>	1.06	1.08	1.07	0.71
	<i>R. monacha</i>	1.03	1.08	1.06	0.94
	<i>C. fumiferana</i> (<i>P. glabra</i>)	1.03	1.09	1.06	0.95
	<i>P. sociata</i>	1.04	1.05	1.04	1.10
	<i>M. americanum</i>	1.04	1.11	1.07	1.34
	<i>M. distorta</i>	1.02	1.08	1.05	1.36
	<i>B. mori</i>	1.04	1.11	1.07	1.34
	<i>C. f. erythrae</i>	1.08	1.11	1.09	1.35
	<i>M. scutiger</i>	1.07	1.09	1.07	1.67
	Capsules	<i>C. muscinana</i>	1.05	1.11	1.07
<i>C. fumiferana</i>		1.01	1.12	1.05	1.87
Ox spleen DNA*		1.04	1.02	1.03	1.22

* Included for comparison; methylcytosine has been added to cytosine.

Composition of beef spleen DNA as determined after two methods of hydrolysis.

Molar ratios calculated to total 3.95, making 4.00 when 0.05 is added for 5-methyl cytosine.

	HCOOH (Mean of 11 ^{analyses} _{estimations})	HCO ₄ (Mean of 9 analyses)
A	1.130 ± 0.008	1.141 ± 0.013
T	1.115 ± 0.006	1.042 ± 0.018
G	0.854 ± 0.007	0.922 ± 0.009
C	0.851 ± 0.009	0.844 ± 0.004

<u>Pd</u>	A			G			C			T		
12-ii	.849	2	4	1.222	1	1	1.123	8	64	.806	7	49
2-iv	.831	16	256	1.218	5	25	1.140	9	81	.809	10	100
19-vi	<u>.861</u>	14	<u>196</u>	1.228	5	<u>25</u>	1.130	1	<u>1</u>	.782	17	<u>289</u>
	3)2.541	6)456		3)3.668	6)51		3)3.393	6)1463		3)2.397	6)438	
	0.847	76		1.223	8		1.131	24		0.799	73	
	± 0.009			± 0.003			± 0.006			± 0.009		

Bm 11-iv	1.174	3	9	0.899	0	0	0.806	2	4	1.118	4	16
2-vi	1.152	19	361	0.908	4	16	0.800	8	64	1.146	24	576
3-viii	<u>1.186</u>	15	<u>225</u>	0.896	3	9	0.817	9	81	1.101	21	441
	3)3.512	6)595		3)2.698	6)25		3)2.423	6)149		3)3.365	6)1033	
	1.171	99		0.899	4		.808	25		1.122	172	
	± 0.010			± 0.002			± 0.006			± 0.013		

Cpe 2-iv	1.170	15	225	0.909	11	121	0.824	13	169	1.099	6	36
2-vi	1.175	10	100	0.901	3	9	0.813	2	4	1.180	5	25
3-viii	<u>1.211</u>	26	<u>676</u>	0.883	15	225	0.797	14	196	1.107	2	4
	3)3.556	6)1001		3)2.693	6)355		3)2.434	6)369		3)3.316	6)65	
	1.185*	166		0.898	59		0.811	61		1.105*	11	
	± 0.013			± 0.008			± 0.008			± 0.003		

Cm 13-iv	1.280			0.791			0.712			1.218		
20-iv	<u>1.294</u>			<u>0.761</u>			<u>0.690</u>			<u>1.252</u>		
	2)2.574											
	1.287			0.776			0.701			1.235		

Ns 20-v	1.270			0.746			0.736			1.246		
19-vi	<u>1.312</u>			<u>0.822</u>			<u>0.694</u>			<u>1.171</u>		
	2)2.582			2)1.568			2)1.430			2)2.417		
	1.291			0.784			0.715			1.208		

Reactions in BSNA by $HClO_4$ hydrolysis.

	Date	% NA in $HClO_4$	Hydrolyzed notes.	A	G	OD	C	T	P recovery
Cantab	25-vii-50	10	72% 1hr BWB	1.096	0.886	(62)	0.846	1.137	101
	12-ix-50	8	72% 1hr BWB	1.230	0.896	(41)	0.860	1.017	98
	17-ix-50	16	72% 1hr BWB	1.16	0.93	(102)	0.84	1.02	101
	19-x-50	11	72% 1hr BWB	1.121	0.898	(102)	0.845	1.083	91
200	14-xii-50	7	70% 1hr BWB	1.13	0.97	(41)	0.840	1.00	
	24-i-51	6.3	"	1.12	0.935	(24)	0.85	1.04	102
	21-vi-51	8	70% 2hrs 97°	1.121	0.938	(41)	0.827	1.113	
	31-xi-51	10	" 100°	1.16	0.95	(41)	0.84	1.00	
	"	20	" 100°	1.15	0.92		0.87	1.00	

Mean
 1.143 0.924 0.846 1.044
 ±0.013 ±0.009 ±0.004 ±0.018

Correct for total
 1.141 0.922 0.844 1.042

Cantab	12-ix-50	$HCOOH$	1.156	0.877	0.871	1.099	89
	17-ix-50	$HCOOH$	1.14	0.85	0.855	1.10	96
	19-x-50	$HCOOH$	1.127	0.837	0.869	1.117	90
200	24-i-51	$HCOOH$	1.11	0.87	0.85	1.11	98

Mean
 1.133 0.86 0.861 1.106
 0.858

Previous mean

BSHA-11007H

A

G

C

T

1.156	23	529	0.877	19	361	0.871	10	100	1.099	7	49
1.14	7	49	0.85	9	64	0.855	6	36	1.10	6	36
1.127	6	36	0.837	13	169	0.869	8	64	1.117	11	121
<u>1.11</u>	<u>23</u>	<u>529</u>	<u>0.87</u>	<u>12</u>	<u>144</u>	<u>0.85</u>	<u>11</u>	<u>121</u>	<u>1.11</u>	<u>4</u>	<u>16</u>
		12)1143			12)738			12)321			12)222

New series

1.133		954	0.858		61	0.861		27	1.106		19
± 0.010			± 0.008			± 0.005			± 0.004		

Old series

1.129	± 0.006		0.851	± 0.006		0.842	± 0.011		1.126	± 0.008	
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Overall mean

1.131	± 0.008		0.855	± 0.007		0.852	± 0.009		1.116	± 0.006	
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Correct for total

1.130			0.854			0.851			1.115		
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~~1.130~~

Test of hydrolysis in BSNA.

Date	A	T	G	C	P recovery	N recovery.
NA 1 hr 5-x	1.133	1.054	0.932	0.826	-	98.8
17-x	1.126	1.091	0.906	0.824	94.1	96.7
20-x	<u>1.143</u>	<u>1.024</u>	<u>0.933</u>	<u>0.844</u>	<u>95.6</u>	<u>95.4</u>
	1.134 ₅	1.056 ₉	0.924 ₅	0.831 ₂	94.9	97.0

NA

NA 2 hrs 5-x	1.146	1.020	0.938	0.843	-	100.0
17-x	1.140	1.061	0.924	0.822	99.3	94.1
20-x	<u>1.144</u>	<u>1.055</u>	<u>0.928</u>	<u>0.822</u>	<u>97.5</u>	<u>95.9</u>
	1.143 ₄	1.045 ₆	0.930 ₁	0.829 ₃₀	98.4	96.6

⁺ Prot 1 hr 5-x	1.115	1.070	0.932	0.831	-	-
13-x	1.113	1.064	0.931	0.840	96.9	-
17-x	1.119	1.147	0.874	0.811	99.0	-
20-x	<u>1.129</u>	<u>1.061</u>	<u>0.916</u>	<u>0.842</u>	<u>100.4</u>	-
	1.119	1.086	0.913	0.831	98.6	-

⁺ Prot 2 hrs 5-x	1.100	1.081	0.926	0.836	-	-
13-x	1.117	1.052	0.936	0.846	98.1	-
17-x	1.117	1.100	0.902	0.830	102.0	-
20-x	<u>1.141</u>	<u>1.089</u>	<u>0.876</u>	<u>0.842</u>	<u>101.2</u>	-
	1.119 ₂₀	1.080 ₁	0.910 ₁	0.838 ₉	100.4	-

Introduction

Discovery of variation in KA's.

Significance?

Genus *Populina* & *Bezdolii*

Materials & Methods

Prep'n of p. & virus. Purification of the viruses & cells.

Chromatographic techniques

Hydrolysis. Results = BSMA. Importance of

inorganic anhydrides.

(1) Temp.

(2) Conc. in HCl.

(3) Added prod.

(4) Carbon residues.

Results

Table of ratios

Lack of result.

Table of constant ratios

Discussion Significance of lack of RNA

VTMV results & animal KA's. Concept of gene-specific KA's

✓ Difference of 2 viruses in one host.

✓ Sulfuric DNA, attempt = gaffing method.

✓ Agreement = isolated LdVHA

✓ Value in identifying viruses.

Insert KA's

Bur 9-xi-49

Kornat 21-xi

Ld 13-xi

Dp 13-xii

Kornat 13-xii

.. 16-iii-50

Lm 24-iv-50

Eckhorn 21-iii-50

Ld 19-v-50

Bur 31-vii-50 474 g → no yield.

234 g → 2.4 mg.

Mean nucleotide residue weights.

	Pd	Lm	Pcf	P ₃	M _a	M _d	B _m	Cpe	Cm	Ns	CCF	BSNA
												mc 39
A (313)	664	771	776	836	915	915	918	936	1000	1010	1026	870
T (304)	609	724	730	781	851	866	851	839	927	921	985	819
G (329)	1002	882	899	804	740	721	740	737	648	642	606	747
C (289)	816	714	708	671	584	586	554	581	514	517	474	604
	3091	3091	3093	3092	3090	3088	3093	3093	3089	3090	3091	3069

A+T/G+C ratios, re-calculated as fraction \pm N + D constant of 40.0.

0.71	16.6 : 23.4
0.945	19.4 : 20.6
1.10	21.0 : 19.0
1.35	23.0 : 17.0
1.67	25.0 : 15.0
1.87	26.1 : 13.9

~~cannot be fortuitous must have some significance~~

	A+T	d	G+C	d		
Pd	41.25	7.35	58.75	7.75		
Lm	48.4	48.6	51.5	51.3		
PCF	48.8		3.8		51.2	3.7
Pb	52.4	5.0	47.6	5.1		
Mg	57.2	57.4	42.7	42.5		
Ma	57.7		5.2		42.2	4.9
Bm	57.3		42.7			
Cpe	57.5		42.5			
Ns	62.6	62.6	37.4	37.4		
CMh	62.6		2.6		37.5	2.6
CCF	65.2		34.8			

	Ratio	d	% increase
83: 97 ¹¹⁷	1	0.71 : 1	11
94: 109	2	[0.83 : 1]	12
99: 103	3	0.94 : 1	16 13
105: 95	4	1.10 : 1	12 14
110: 90	5	[1.22 : 1]	13 14
115: 85	6	1.35 : 1	15
120: 80	7	[1.50 : 1]	17
125: 75	8	1.67 : 1	20
130: 70	9	1.87 : 1	

$$8 \overline{) 53.5} \\ \underline{6.69}$$

$$6.69\% = 1 \text{ in } 15$$

$$\frac{40}{200}$$

$$71:100$$

$$71 \times \frac{40}{171}$$

$$\frac{A+T}{G+C} = 0.71$$

$$A+T = 0.71 (G+C)$$

$$A+T = 0.94 (G+C)$$

$$A+T = 1.10 (G+C)$$

$$\text{Total} = 1.71$$

$$1.94$$

$$2.10$$

$$\begin{array}{r} 221 \\ 283 \\ 264 \\ \hline 310 \end{array}$$

$$0.94 \times \frac{4}{194}$$

—

///
—

—

CP

CM

MS

- - - - -

—

CP

MS

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— — —

	A	T	G	C	
Bm 1-xi-49 ^{Corr. for RNA}	1.11	1.20	0.87	0.82	
Lm 24-iv-50	1.44	1.43	0.56	0.68	
Carlson 21-iii-50	1.51	1.03	0.60	0.95	
<hr/>					
Pd V new	0.848	0.802	1.220	1.130	4.000
PLVNA old	0.86	0.81	1.20	1.12	

$$\frac{3}{195}$$

$$\frac{3}{162}$$

Properties of Insect Viruses.

Designation	Food	Virus name	Type of inclusion body
Pd	Gypsy moth (<i>Portetia</i> <i>lapar</i> (R.); <i>Lepidoptera</i> , <i>Lymantriidae</i>)	<i>Borrelia reformans</i> Holmes	Polyhedron
Lm	Worm moth (<i>Lymantria</i> <i>monacha</i> L.; <i>Lepidoptera</i> , <i>Lymantriidae</i>)	<i>Borrelia efficiens</i> Holmes	Polyhedron
Bm	Silkworm (<i>Bombyx mori</i> L.; <i>Lepidoptera</i> , <i>Bombycidae</i>)	<i>Borrelia bombycis</i> Paillot	Polyhedron
Cpe	Alfalfa butterfly (<i>Colias philodice</i> <i>eurytheme</i> Boisd.; <i>Lepidoptera</i> , <i>Pieridae</i>)	<i>Borrelia saundersi</i> Standen	Polyhedron
Ma	Orchard tent caterpillar (<i>Melanocoma</i> <i>americanum</i> (Fabr.); <i>Lepidoptera</i> , <i>Lasiocampidae</i>)	—	Polyhedron
Md	Forest tent caterpillar (<i>Melanocoma</i> <i>disstris</i>)	—	Polyhedron