nous supply of IAA promotes root formation. Thus IAA oxidase does not appear to play merely a detoxifying role. It may be that IAA oxidase enzyme acts on IAA to produce some oxidative products which are biologically more active than IAA[13-16]; however, there are many reports contrary to this postulate[17-19]. The 3rd possibility is that IAA oxidase and phenolase enzymes act on IAA and some 'phenolic co-factors' respectively modifying them in such a way as to form 'auxin-phenolic conjugates', which cause/promote the formation of adventitious roots[20-22]. That inhibition of activity is caused by the inhibitor(s) present in the protein extract of *E. citriodora* which also inhibits the formation of roots on cuttings of *Phaseolus mungo* is rather interesting. However, more work is needed to check on this point.

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DNA sequence organisation in relation to genome size in birds[1]

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Summary. DNA sequence interspersion was investigated in pheasant and pelican nuclear DNA. As is typical for birds, these genomes are organized in a long period pattern. Altogether, 5 bird species with genome sizes between 1.6 and 1.9 pg DNA are compared with regard to the extent of repetitive and single copy sequence interspersion. The result indicates that the average length of interspersed repetitive sequences increases with genome size.

A consistent feature of eukaryotic nuclear DNA is the presence of repeated sequences[4]. In many genomes, short repetitive sequences of about 0.3 kilobases (kb) are regularly interspersed with unique sequences less than 2 kb in length[5]. This type of sequence organisation has been termed 'short period interspersion' pattern, or 'Xenopus-pattern', after the organism in which it was first described in detail[6]. The functional, or structural significance, if any, of this pattern is not known, but among others regulatory tasks have been ascribed to it[7]. However, in a wide range of organisms, this pattern appears to be absent, or reduced beyond detectability by the methods commonly used so far, i.e. DNA reassociation techniques[5]. Such genomes are said to be organized in a 'long period interspersion' pattern, that is, repetitive and unique sequences extend uninterrupted for several kb each, an example being the genome of *Drosophila*[8]. It has been noted that a correlation seems to exist between the presence of short period interspersion and genome size: if, among related species with differing nuclear DNA content, genomes with and without short period interspersion co-exist, it is usually the smaller genomes in which this pattern is not detectable[5,9,10]. It is conceivable that genomes organized in the long period fashion are derived from *Xenopus*-like genomes in the course of evolution, through loss and rearrangement of repeated sequences[11].

Among the vertebrates, the only group of species in which short period sequence interspersion is usually absent are birds[12,13]. Compared with their closest relatives, birds have smaller genomes: reptile genomic DNA contents range between 60 and 89% of the mammalian value, whereas bird genomes have 44–59%[14]. We have observed, however, that among birds there seems to be an inverse correlation between genome size and the amount of sequence interspersion on a given stretch of DNA[13]. This correlation was based on observations of 3 species only, chicken *(Gallus domesticus),* pigeon *(Columba livia domestica),* and duck *(Cairina domestica).* We have now investigated the mode of DNA sequence interspersion in 2 additional bird nuclear genomes, namely those of the pheasant *(Phasianus colchicus)* and the pelican *(Pelicanus occidentalis).* These results are reported here, and are discussed together with the previous findings.

Materials and methods. Pheasants were obtained from a local poultry hatchery. Pelican blood was obtained with the kind cooperation of Tierpark Hellabrunn (München) and Vogelpark Walsrode. DNA was extracted from blood cell nuclei as described before[13]. The mode of sequence interspersion in pheasant and pelican nuclear DNA was evaluated by comparing the reassociation of long and short fragments, using conventional techniques[15]. When using the hydroxyapatite assay to monitor reannealment as a function of incubation time, this method directly reveals the proximity of sequences with different repetition frequencies. In this assay DNA molecules which are fully or only partially double stranded become absorbed to HAP at low salt concentration. Thus, with a high degree of repetitive and unique sequence interspersion, more DNA is retained

Kinetic analysis of pheasant and pelican DNA

		Very fast		Fast		Slow	
	Fragment length (kb)	Fraction size (%)*	Rate (M^{-1} sec^{-1})	Fraction size (%)*	Rate (M^{-1} sec^{-1})	Fraction size (%)*	Rate $\times 10^3$ (M^{-1} sec^{-1})
Pheasant	0.2	12.5±1.4	$> 10^4$	7.2±2.3	1.13±1.79	80.3±2.4	1.4±0.2
	2.1	15.8±1.2	$> 10^4$	19.3±3.4	0.50±0.32	64.9±3.4	6.5±1.3
Pelican	0.21	14.8±1.1	$> 10^4$	15.3±5.7	00.37±0.032	69.9±5.2	0.9±0.2
	2.0	18.6±3.7	$> 10^4$	9.9±3.7	277±343	71.5±1.7	4.0±0.5

* Corrected for non-reactive material.

on HAP at a given Cot using long fragments than using short fragments. Thus, the amount of DNA lost from the fraction renaturing with single copy properties using long versus short DNA can be used as an index of sequence interspersion.

Results and discussion. The reassociation kinetics of long and short DNA fragments of pheasant and pelican DNA are shown in figure 1, and are evaluated in the table. A comparison of the analytical genome size determination (see legend to fig. 2) with the kinetic results (using *E. coli* DNA as a standard (0.0047 pg[16], Cot $1/2 = 2.6$[12])) shows that the slowest kinetic fractions of the short fragment curves are composed of unique sequences. When the starting fragment length is raised about 10-fold, an approximately 3-fold (square root of fragment length ratio) acceleration of the reassociation rate is expected[15]. The observed values are in accordance with the expectation. Thus, in the long fragments kinetics, the slowest fraction also consists of unique sequences, within the limits of accuracy of the method employed. Depending on the degree of sequence interspersion, the single copy fraction is expected to decrease in size with increasing fragment length, as explained in the materials and methods section. In the case of pheasant DNA, the single copy fraction is 15% smaller on long fragments than on short ones. The moderately repetitive fraction is increased by almost this amount, while the highly repetitive component has increased by a few percent only. This result demonstrates that moderately repetitive sequences are interspersed to a certain extent with unique sequences, and that highly repeated sequences are interspersed with unique and/or moderately repeated sequences, on the fragment length used. It is emphasized, however, that the extent of sequence interspersion is much smaller than that known from *Xenopus*-like organized DNA[17]. The degree of single copy and repeated sequence interspersion in pheasant DNA is similar to that observed in the chicken genome, which has been shown to be organized in a long period fashion, using a variety of methods[12, 18, 19]. In pelican DNA long period sequence arrangement is even more pronounced. The amount of sequences reassociating with single copy properties is prac-

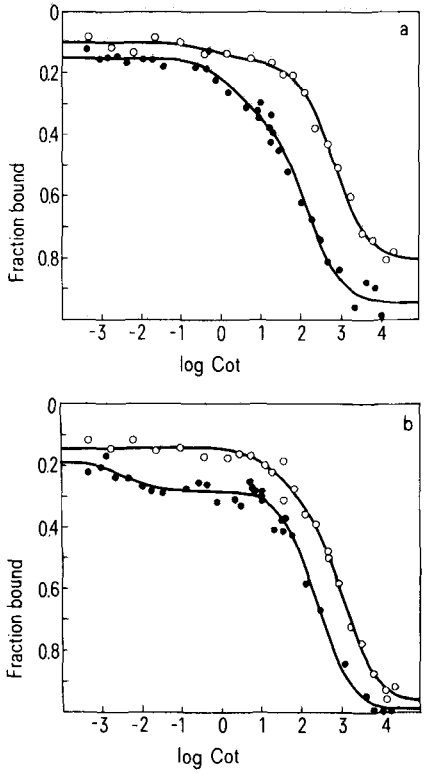

Figure 1. DNA reassociation kinetics of *a* pheasant and *b* pelican nuclear DNA. Open symbols refer to short (0.2 kb) fragments, filled symbols refer to long (2.1 kb, pheasant; 2.0 kb, pelican) fragments. Fraction bound indicates the fraction of the DNA bound to hydroxyapatite at a given value of Cot (normalized to 0.12 M phosphate buffer[15]) (Cot is the product of DNA concentration (moles of nucleotides/l) and time (sec)). Fragment lengths were determined by alkaline sucrose gradient centrifugation[12]. The computer-aided least squares analyses of the data points for 3 second order kinetic components are given in the table.

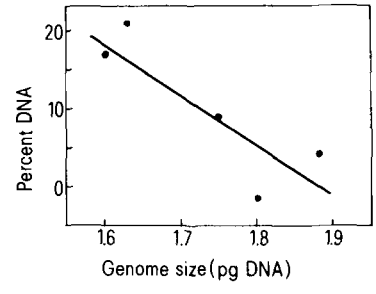

Figure 2. The extent of repetitive and unique sequence interspersion in bird genomes of different nuclear DNA content. The percentages of DNA reassociating with single copy properties on short (0.2–0.25 kb) minus that on long (2.0–2.7 kb) fragments are plotted as a function of genome size. The interspersion data are combined from previous reports[12, 13] and the results from the present study. Genome sizes were determined by Feulgen cytophotometry: *Phasianus colchicus* 1.6 pg, *Gallus domesticus* 1.63 pg, *Cairina domestica* 1.75 pg, *Pelicanus occidentalis* 1.8 pg, *Columba livia domestica* 1.88 pg. It is seen that a progressively smaller portion of single copy DNA is adjacent to repeated sequences on the fragment length used, with increasing genome size. The data fit to a linear relationship ($r = 0.8969$).

tically unaltered when the starting fragment length is increased from 0.2 to 2 kb. Here, intermediately repetitive sequences seem to be interspersed only with highly repeated ones.

As shown in figure 2, the previously suggested inverse correlation between the extent of sequence interspersion and genome size in bird nuclear DNA is confirmed. With increasing genome size, from 1.6 to 1.9 pg DNA per haploid genome, less single copy DNA appears to be adjacent to repeated sequences on 2–2.7 kb long fragments. Since the absolute amount of unique DNA shows no significant increase with increasing genome size, our result is interpreted as reflecting an increased average length of repetitive sequences, neighbouring single copy sequences in the larger genomes. It is not possible to say whether the genome size variations in bird genomes are due mainly to additions or loss of repetitive sequences relative to a common ancestral genome. Tandem duplication would serve as a likely mechanism to increase repetitive sequence length. A mechanism reducing repetitive sequence length, on the other hand, if not a totally random process, but rather depending on sequence structure, could be facilitated by repetitive sequence subrepetitivity. The mosaic structure of the long repeats in *Drosophila* DNA, some subrepeats of which are considered as transposable elements[20], may serve as a model for repetitive sequence evolution in birds. There is evidence that the long repeats at least in chicken DNA have a subrepeat composition[21].

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Prostaglandin-like substances in *Propionibacterium acnes*. VI. Characterization of the lipid fraction by gas chromatography in conjunction with mass spectrometry[1]

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Summary. The lipid fraction of *P. acnes* was submitted to stepwise purification followed by bioassay in order to localize the prostaglandin-like material. GC-MS analysis revealed the occurrence of substances having a part but not a total molecule in common with the prostaglandin family, suggesting that prostaglandin-like substances represent a new group of prostaglandin compounds.

Our investigations on the lipids of *Propionibacterium acnes* revealed the occurrence of prostaglandin-like substances (PLS) in these bacteria[2]. Furthermore, we could demonstrate that these substances mimic E-prostaglandins in various biological situations in vitro (gerbil colon bioassay[3], human blood vessels[4]) or in vivo (hamster cheek pouch). PLS induces an acceleration of cyclic AMP synthesis in rat ovaries[5] and possesses significant chemotactic properties[6]. In spite of great similarities in the biological response between PGE_2 and PLS, our results point to the fact that PLS seems to differ from PGE_2. This was especially apparent in the bioassay with a human utero-tubal junction[7] as well as in cascade superfusion bioassay[8]. Moreover, PLS also demonstrated a potent inhibitory effect on human platelet aggregation[8]. In order to establish whether PLS contain PGE_2, investigations with reversed-phase chromatography and gaschromatography-mass spectrometry were performed[9]. These analyses could clearly demonstrate that PLS were not identical with PGE_2.

This investigation is an attempt to determine the structural composition of the lipid fraction from *P. acnes* with special reference to the localization of PLS.

Material and methods. PLS from *P. acnes* were extracted as described in an earlier communication[2]. In the first purification step prostaglandin-like compounds were separated from other lipids via dry-column chromatography (Woelm). As large samples were necessary for further work, we designed a new dry-column system (unpublished data). This system allows a rapid work-up with a minimum of losses. A solvent system I[10] was used. The biologically active fraction was then submitted to preparative TLC. An automatic liner developed in our laboratory, allowing rapid narrow-band application of samples dissolved in large solvent volumes, was used to apply the samples to precoat-