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Investigation of the Influences of Puberty, Genetics, and Environment on the Composition of Subgingival Periodontal Floras

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The classical twin model was utilized in this study in an attempt to determine the importance of host genetics to the composition of the subgingival flora. Simultaneously, the effect of puberty on the flora composition was assessed. The compositions of the floras were significantly different at ages 11 and 14 in the same people, indicating that transition to an adult flora composition may be initiated during puberty. However, the numbers of subjects who had prepubertal and postpubertal testosterone levels in this study were too small to demonstrate significant differences based solely on testosterone level ($P = 0.053$ and 0.11 for tests of unrelated members, i.e., all twins "a," the first twin of each pair, and all twins "b," the second twin of each pair). Sixteen unrelated 11-year-old subjects had prepubertal levels of less than 30 ng of testosterone per dl of serum, and only six of these unrelated subjects had levels above 300 ng/dl by age 14. Of their twin siblings, who formed the second group of unrelated individuals, 15 had prepubertal levels and only 5 reached postpubertal levels. Unpaired *t* tests indicated that *Veillonella atypica*, *Prevotella denticola*, and *Prevotella melaninogenica* were among the species that contributed most to changes in flora composition during puberty. The compositions of subgingival floras of 11-year-old monozygous and dizygous male twins were significantly more similar than those of unrelated subjects in the study ($P = 0.004$ and 0.009, respectively). At 12.5 years of age, the floras of monozygous twins remained more similar than those of unrelated subjects ($P = 0.001$), but the dizygous-twin floras were not significantly more similar than those of unrelated people. This difference corresponded with moderate and varied testosterone levels within dizygous-twin pairs at age 12.5. By age 14 both monozygous and dizygous twins again had floras with compositions more similar than those of unrelated people ($P = 0.008$ and 0.002, respectively). Estimates of the genetic contributions to the increased similarity of the floras of twins as compared with floras of unrelated people indicated that the concentrations of several species in the flora may be influenced by host genetic factors. The prevalence of certain other species appeared to be controlled primarily by environment.

Previous studies have shown that a major source of variation in the composition of the human subgingival flora is the individual subject within any population group or disease category of patients (4, 27). Similar person-to-person variation was demonstrated earlier in studies of the human intestinal flora (3, 13, 25). It appears that each individual tends to maintain his or her own type of flora, and attempts to permanently modify individual flora composition usually are unsuccessful (3, 5, 25, 34). Large person-to-person variation in composition of the bacterial flora is the major reason that numerous subjects are required to obtain statistically significant results in studies of populations or of human diseases that may relate to indigenous floras.

Previous studies, mostly cross-sectional, also have indicated that the composition of the periodontal flora of children differs from that of adults (1, 2, 4, 6, 9, 10, 16, 28, 35–37). Of these, only two reports (9, 36) were of longitudinal studies that extended for more than 1 year, and subjects in both of those studies were unrelated. From studies which utilized the experimental gingivitis model, Moore et al. (28) reported that the associated floras of four 4-year-old children differed significantly from the associated floras of four 22- to 31-year-old adults. However, in a later study they found that the floras of 10 4- to 6-year-old children with naturally occurring gingivitis were not significantly different ($P = 0.85$) from the floras of 11 24- to 34-year-old adults with naturally occurring gingivitis.

A possible hormonal effect on flora composition was suggested by Kornman and Loesche (19), who reported a positive correlation between black-pigmented *Bacteroides* spp. and serum levels of estradiol in pregnant women. Gusberti et al. (9) studied subgingival flora composition in relation to pubertal development. They reported that the frequencies of *Actinomyces odontolyticus* and *Capnocytophaga* sp. increased with age in both males and females and that the frequencies of *Prevotella* (*Bacteroides*) *intermedia* and *Prevotella* (*Bacteroides*) *melaninogenica* in the male subjects increased during early puberty.

It would be advantageous to know when individuals attain their adult floras and why individuals tend to maintain their own distinct flora compositions. If the flora composition is controlled primarily by genetic means (e.g., host compatibility with certain floras based on inherited immune capabil-

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ity, physiology, metabolism, mucus composition, or receptor-ligand interactions), then it should be possible to predict which individuals may be at greatest risk for diseases that relate directly to the flora of mucous membrane surfaces. If, on the other hand, control of flora composition is primarily a response to environment (e.g., initial bacterial colonization and initial immune responses that in turn direct subsequent flora development, nutrition, or hygiene), then it should be possible to modify normal floras (either during initial colonization or perhaps later) to produce greater resistance to certain diseases.

In an attempt to determine when individuals attain their adult floras relative to hormonal changes and to determine the relative effects of genetics and environment on flora composition, we examined the subgingival floras of monozygous (MZ) and dizygous (DZ) twins at 11, 12.5, and 14 years of age, when hormonal changes can be expected to occur. This constitutes the first report of microbiological studies of the periodontal floras of twins between the ages of 11 and 14 years.

MATERIALS AND METHODS

Subjects. Ten pairs of 11-year-old male MZ twins and ten pairs of 11-year-old male DZ twins were sampled for bacteriological studies, zygosity determinations, and measurement of blood testosterone levels. The subjects were medically healthy and had not taken antibiotics within 60 days of sampling. Nine pairs of MZ twins returned for bacterial and blood samples at 12.5 and 14 years of age. Eight pairs of DZ twins returned for sampling at 12.5 years of age, and seven returned at 14 years of age.

At each sample time each subject was monitored clinically for probeable attachment level at four sites per tooth (mesiobuccal, midfacial, distobuccal, and midlingual sites). In addition to pocket depth and cemento-enamel junction determinations to derive probeable attachment level, each subject was assessed for plaque index (31), gingival index (21), bleeding index (30), gingival suppuration (32), and tooth mobility.

Zygosity was determined by questionnaire, dermatoglyphic analyses, and testing of the twins and their parents for the ABO, Rh, MNS, Kell, Fy, JK, and P blood group systems by standard methods.

Blood testosterone levels were determined by radioimmunoassay (Nicholas Institute, San Juan Capistrano, Calif.) (18). Levels were considered as follows: <30 ng/dl, prepubertal; 30 to 100 ng/dl, early pubertal; 100 to 300 ng/dl, late pubertal, and >300 ng/dl, postpubertal.

Bacteriological samples. Subgingival samples of the periodontal flora were taken for bacteriological study from two to four subgingival sites (usually the mesial surface of permanent first molars, teeth 3 and 19 or teeth 14 and 30) in each subject at each sample time. Samples were taken from the depth of the gingival crevice by a single pass with a sterile Morse 00 scaler, diluted, and cultured anaerobically as described previously (29). A total of 30 isolated colonies was picked from each sample in a randomized pattern from anaerobic roll tubes and blood agar plates (28). Pure cultures was characterized by biochemical tests (12, 23), polyacrylamide gel electrophoresis of soluble proteins (26), and cellular fatty acid composition (7, 22).

Statistical analyses. Good's lambda analysis (8, 29), based on minimum percent similarity (33), and Student's unpaired *t* test were used to compare the compositions of the floras and to compare the relative incidences of individual species in the samples.

A combined single pair of samples from each subject was used for lambda analysis (8, 29) to compare the similarity of the floras of twins with that of the floras of unrelated subjects. For these comparisons, one twin of each pair was arbitrarily assigned to group "a" of unrelated subjects and the other was assigned to group "b" of unrelated subjects. Combined single pairs of samples (60 isolates, representing 30 from each of two sites) from each subject also were used for comparisons of the twins' floras, at different ages, with floras of adult and child populations studied previously and with floras of subjects who had naturally occurring gingivitis (24). Sites from the subjects with naturally occurring gingivitis were chosen, insofar as possible, to have gingival index scores similar to those observed in the twin population. The mean gingival index scores were 0.85 in children with naturally occurring gingivitis, 0.86 in adults with naturally occurring gingivitis, 0.92 in 11-year-old a twins, 0.75 in 11-year-old b twins, 1.06 in 14-year-old a twins, and 1.09 in 14-year-old b twins.

Heritability of the quantitative occurrence of each bacterial species (considered as a "trait") was estimated by using arc sine transformations of the percentages of the flora of each subject that were represented by each species. One estimate was by the unweighted least-squares formulas of Haseman and Elston (11), which utilize transformed data from both MZ and DZ twins simultaneously. Heritability also was estimated as proposed by Kempthorne and Osborne (17), using analysis-of-variance tables based on the individual observations for each twin in which arc sine-transformed percentages of species from MZ twins and from DZ twins were analyzed separately. The results from DZ twins provided an estimate of heritability in the narrow sense.

TABLE 1. Gingival index scores of sampled sites

Parameter	Mean gingival index score (no. of sites)		
	MZ	DZ	MZ + DZ
Age (yr)			
11	0.79 (48)	0.92 (64)	0.87 (112)
12.5	1.00 (58)	1.18 (54)	1.09 (112)
14	1.11 (72)	1.11 (44)	1.11 (116)
Testosterone level (ng/dl)			
ND[a]	(4)	(8)	(12)
0–30	0.79 (68)	0.94 (82)	0.87 (150)
31–100	1.16 (38)	1.04 (24)	1.11 (62)
101–300	1.14 (36)	1.19 (36)	1.17 (72)
>300	1.03 (32)	1.33 (12)	1.11 (44)

[a] ND, not determined.

RESULTS

Clinical measurements. Summary results of the clinical assessments have been reported (3a). Briefly, both gingival index (Table 1) and bleeding index scores for the combined MZ and DZ boys increased with time, as did levels of testosterone. By both intraclass correlation and Pearson's correlation, the similarities of testosterone levels were significantly more correlated for MZ twins than for DZ twins over the study period. Plaque index and gingival index scores were of equal similarity between MZ and DZ twins,

TABLE 2. Effect of age and testosterone level on the composition of periodontal floras as determined by Good's lambda analysis of samples combined from two sites to represent the flora of each individual at each time

Comparison	P	
	Twins a	Twins b
Age, yr (*n*)		
11 (20) vs 12.5 (17)	0.77	0.54
12.5 (17) vs 14 (16)	0.43	0.006
11 (20) vs 14 (16)	0.007	0.001
Testosterone, ng/dl (*n*)		
<30 (15[a]) vs >300 (5)	0.11	
<30 (16[a]) vs >300 (6)		0.053

[a] All were age 11.

but over the time studied MZ twins were more similar than DZ twins for bleeding index scores.

Flora composition. The compositions of the floras of the subjects were significantly different at 11 versus 14 years of age (Table 2). There was no significant difference in the compositions of the floras at ages 11 and 12.5 for either twin set. The compositions of the floras of the b twins, but not those of the a twins, were significantly different at 12.5 and 14 years.

There was no significant difference between the compositions of the floras of individuals with testosterone levels of <30 ng/dl and the compositions of the floras of those with testosterone levels of >300 ng/dl in the 15 versus 5 and 16 versus 6 unrelated individuals compared, although the values for the b twins approached significance ($P = 0.053$). In order to prevent bias that might result from using some of the same people more than once, values for 12.5-year-olds were not used in the comparisons of floras associated with different testosterone levels.

Unfortunately, only 50% of the MZ twins and 21% of the DZ twins attained adult levels of testosterone (>300 ng/dl) by age 14 (Table 3). Fifty samples were from individuals with testosterone levels of <30 ng/dl, 30 were from subjects with testosterone levels of 30 to 300 ng/dl, and 11 were from individuals with testosterone levels of >300 ng/dl. Although there was an increase in the mean testosterone levels with age, variation among individuals was high at ages 12.5 and 14.

To determine the nature of the differences in flora composition, an unpaired *t* test was done for all species in the floras of the subjects at ages 11 and 14 (Table 4). Results for a and b twins of the pairs were analyzed separately to avoid bias from different numbers of MZ and DZ samples. Because no statistical penalty was paid for comparisons of over 100 species (that is, the *P* values were not adjusted to account for the fact that multiple comparisons were made), the results in Table 4 may not be remarkable except for *Veillonella atyp-*

TABLE 3. Testosterone levels of test subjects and number of periodontal sites sampled

Twin	Testosterone, ng/dl (no. of sites)					
	11-yr-olds		12.5-yr-olds		14-yr-olds	
	MZ	DZ	MZ	DZ	MZ	DZ
1a	10 (2)	ND[a] (4)	31 (4)	ND (2)	560 (4)	79 (4)
1b	18 (2)	37 (4)	54 (4)	ND (2)	412 (4)	171 (4)
2a	ND (2)	9 (4)		28 (4)		77 (4)
2b	ND (2)	25 (4)		174 (4)		382 (4)
3a	5 (2)	10 (4)	8 (4)	21 (2)	29 (4)	270 (4)
3b	4 (2)	4 (4)	6 (4)	18 (2)	27 (4)	238 (4)
4a	9 (2)	5 (4)	26 (2)	204 (3)	82 (4)	321 (4)
4b	13 (2)	3 (4)	38 (2)	160 (3)	366 (4)	336 (4)
5a	63 (2)	5 (2)	273 (4)	24 (4)	637 (4)	45 (2)
5b	32 (2)	5 (2)	290 (4)	13 (4)	530 (4)	179 (2)
6a	55 (2)	12 (4)	155 (2)	117 (4)	339 (4)	196 (2)
6b	74 (2)	8 (4)	239 (2)	78 (4)	334 (4)	171 (2)
7a	8 (2)	9 (2)	84 (4)	92 (4)	320 (4)	
7b	7 (2)	11 (2)	163 (4)	18 (4)	294 (4)	
8a	9 (2)	3 (2)	12 (2)		21 (4)	
8b	10 (2)	4 (2)	8 (2)		41 (4)	
9a	13 (4)	7 (4)	38 (4)	16 (4)	254 (4)	159 (2)
9b	20 (4)	5 (4)	63 (4)	22 (4)	266 (4)	219 (2)
10a	8 (4)	39 (2)	16 (3)		152 (4)	
10b	16 (4)	6 (2)	11 (3)		111 (4)	
Mean ± SEM[b]	20.8 ± 5.0	10.9 ± 2.5	84.7 ± 22.7	70.4 ± 18.1	265.3 ± 45.5	203.1 ± 26.8

[a] ND, not determined.

[b] Mean ± standard error of the mean for MZ plus DZ: age 11, 15.7 ± 2.8; age 12.5, 78.1 ± 18.1; age 14, 238.1 ± 26.8.

TABLE 4. Probability values for bacterial species that are significantly associated with different ages

Twins[a]	Total no. of taxa	Associated with 11-year-olds		Associated with 14-year-olds	
		P	Species	P	Species
a	141	0.03	*Actinomyces* serotype WVA 963	0.003	*Prevotella melaninogenica* 2381
		0.03	*Actinomyces gerencseriae*	0.01	*Streptococcus* "SA"
				0.02	*Veillonella atypica*
b	138	0.01	*Capnocytophaga ochracea*	0.001	*Veillonella atypica*
		0.01	*Neisseria mucosa*	0.005	*Prevotella denticola*
		0.03	*Actinomyces israelii* (−)[b]	0.04	*Eubacterium brachy*
		<0.05	*Veillonella parvula*	<0.05	*Streptococcus mutans*

[a] a, represented by one twin of each MZ and DZ pair; b, represented by the second twin of each MZ and DZ pair.
[b] (−), Strains with phenotypic characteristics of *A. israelii* but not reactive with available fluorescent-antibody conjugates.

ica, which was significant in both groups, and for *Prevotella denticola* and *P. melaninogenica* DNA homology group 2381 (ATCC 25845), which have very low probabilities.

Concentrations of actinomyces, particularly *Actinomyces gerencseriae* (15) and *Actinomyces israelii* (−) (see Table 4, footnote *b*), differed in floras of the 11- and 12.5-year-olds (data not shown) and the 11- and 14-year-olds. *Streptococcus* "SA," which was associated with 12.5- and 14-year-olds, is similar to *Streptococcus anginosus* but differs from *S. anginosus* by polyacrylamide gel electrophoresis and cellular fatty acid profiles and by not hydrolyzing esculin and not fermenting salicin. *Capnocytophaga ochracea* and *Neisseria mucosa* were associated with 11- and 12.5-year-olds compared with 14-year-olds in the b twin set. *Streptococcus mutans*, *P. melaninogenica* 2381, *P. denticola*, and *V. atypica* were associated with 14-year-olds. Of these, only *V. atypica* was found at significantly different levels in both a and b sets of twins (11 versus 14 years).

When the floras of the populations of 10 children (ages 4 to 6) and 11 adults (ages 24 to 34) studied previously (24) were reanalyzed by using only two samples from each individual, the floras were not statistically significantly different by lambda analysis ($P = 0.58$). However, the flora of the 11-year-old twins in the present study was statistically significantly different from that of the adults (Table 5), and the flora of the 14-year-old twins was different from those of both children and adults. Species that were significantly different among the children, adult, and twin populations are given in Table 6. Again, no adjustment in *P* values was made as a penalty for testing multiple samples, but the low probability values are more frequent than would be expected by chance among this many comparisons and indicate that the listed species include those that account for major differences in the compositions of the floras of the different groups of people. No species are listed for 11-year-old twins as compared with children ages 4 to 6 because these floras were not statistically different by Good's lambda analysis.

Except for the DZ twins at 12.5 years of age, the compositions of the floras of both MZ and DZ twin pairs were significantly more similar than those of unrelated people (Table 7).

All three heritability estimates yielded positive heritability values associated with several species, to suggest a possible genetic influence on their prevalence (Tables 8, 9, and 10). The prevalence of some other species appeared to be influenced primarily by environmental factors (Table 8), as suggested by negative values by all three heritability estimates. Differences in the species listed at 11 and 14 years of age suggest that the genetic influence may change or may affect different species before and after puberty or may interact with hormone influences. Heritability estimates for other species varied depending on the analytical method used. In 40 subjects at 11 years of age, 142 species were detected among 3,528 isolates. In 34 subjects at 12.5 years of age, 162 species were detected among 3,517 isolates. In 32 subjects at 14 years of age, 166 species were detected among 3,642 isolates.

TABLE 5. Results of lambda analyses comparing floras of 11- and 14-year-old twins with floras of children and adult populations

Population (*n*)	*P*[a]			
	11-yr-old twins		14-yr-old twins	
	Set a (*n* = 20)	Set b (*n* = 20)	Set a (*n* = 16)	Set b (*n* = 16)
Children (20)	0.39	0.53	0.006	0.001
Adults (22)	0.02	0.09	0.01	0.001

[a] Probability based on two samples of 30 isolates each to represent the flora of each subject.

DISCUSSION

The results of this study indicate that the composition of the microflora of 11-year-old boys is like that of younger children but becomes significantly changed by age 14. The composition of the subgingival flora at age 14 also was significantly different from that of adults, indicating that the subjects had not yet attained the adult flora. Moreover, most of the subjects did not have adult levels of testosterone at that age. Only 11 of 100 samples were from individuals who had >300 ng of testosterone per dl of blood by age 14.

A. gerencseriae (formerly *A. israelii* serotype II), *A. israelii* (−), *C. ochracea*, and *N. mucosa* were associated with the floras of the prepubertal children. These observations confirm previous studies (4, 14) that report higher concentrations of *C. ochracea* or all capnocytophagas in children than in adults. However, Gusberti et al. (9) reported a "statistically significant trend of increasing proportions of *Capnocytophaga* (p = 0.01) with chronological age in both sexes."

Our observations do not corroborate the observations of Delaney et al. (4) that levels of actinomyces species were unrelated to all indicators of sexual maturation. Delaney et al. report the isolation of only *Actinomyces naeslundii*, *Actinomyces viscosus*, and *A. odontolyticus*. It is difficult to believe that *A. israelii* and *A. gerencseriae* would not have been isolated on the nonselective medium that they used for

TABLE 6. Probability values for bacterial species that are significantly associated with different populations

Twins[a]	Total no. of taxa	P	Species	P	Species
			Associated with 11-yr-olds		Associated with adults
a	131	0.04	*Actinomyces gerencseriae*	<0.001	*Streptococcus sanguis* I
				0.01	*Wolinella* "X"
				0.02	*Peptostreptococcus micros*
				0.02	*Streptococcus intermedius*
				0.02	*Bacteroides forsythus*
				0.02	*Haemophilus segnis*
				0.05	*Prevotella denticola*
b	139	0.02	*Bacteroides* "D34"	0.01	*Streptococcus sanguis* I
				0.01	*Wolinella* "X"
				0.02	*Bacteroides forsythus*
				0.02	*Streptococcus oralis*
				0.02	*Haemophilus segnis*
				0.02	*Haemophilus parainfluenzae*
				0.02	*Eubacterium timidum*
			Associated with 14-yr-olds		Associated with children
a	129	0.02	*Lactobacillus rimae*	0.005	*Prevotella* "D1C20"
		0.03	*Veillonella atypica*	0.006	*Actinomyces gerencseriae*
				0.02	*Streptococcus intermedius*
b	120	0.02	*Actinomyces naeslundii* III	0.01	*Capnocytophaga ochracea*
				0.01	*Propionibacterium acnes*
		0.02	*Prevotella denticola*	0.03	*Streptococcus intermedius*
		0.03	*Veillonella atypica*	0.03	*Prevotella* "D1C20"
				0.05	*Wolinella recta*
			Associated with 14-yr-olds		Associated with adults
a	145	0.02	*Veillonella atypica*	0.01	*Actinomyces naeslundii* (−)[b]
		0.03	*Streptococcus parvulus*		
		0.03	*Streptococcus* "SA"	0.03	*Wolinella* "X"
		0.04	*Lactobacillus rimae*	0.03	*Streptococcus sanguis* I
		0.04	*Actinomyces georgiae*	0.04	*Actinomyces meyeri*
				0.04	*Bacteroides forsythus*
				0.05	*Actinomyces odontolyticus* (−)[c]
b	138	0.01	*Lactobacillus rimae*	0.006	*Streptococcus sanguis* I
		0.02	*Veillonella atypica*	0.01	*Streptococcus morbillorum*
		0.04	*Streptococcus parvulus*	0.01	*Neisseria mucosa*
		0.05	*Bacteroides* "D34"	0.03	*Wolinella* "X"
				0.04	*Wolinella recta*
				0.04	*Actinomyces meyeri*
				0.04	*Bacteroides forsythus*
				0.04	*Streptococcus mitis*
				0.04	*Haemophilus parainfluenzae*
				0.05	*Actinomyces odontolyticus* (−)

[a] a, represented by one twin of each MZ and DZ pair; b, represented by the second twin of each MZ and DZ pair.
[b] (−), strains with phenotypic characteristics of *A. naeslundii* but not reactive with available fluorescent-antibody conjugates.
[c] (−), strains with phenotypic characteristics of *A. odontolyticus* but not reactive with available fluorescent-antibody conjugates.

TABLE 7. Mean similarity of floras and probability values for the observed greater similarity of compositions of floras of twins compared with floras of unrelated people

Group	Mean similarity ± SEM (*P*)[a]		
	11-yr-olds	12.5-yr-olds	14-yr-olds
MZ twin pair	38.15 ± 3.96 (0.004)	35.10 ± 2.48 (0.001)	32.71 ± 2.32 (0.008)
DZ twin pair	37.21 ± 2.03 (0.009)	29.68 ± 2.37 (0.68)	30.90 ± 3.63 (0.002)
Unrelated	30.44 ± 0.22	26.49 ± 0.25	26.66 ± 0.22

[a] Probability based on lambda analysis using one combined sample of 60 isolates to represent the flora of each subject.

TABLE 8. Estimates showing heritability by each of three tests at age 11

Taxon	Heritability value[a] (no. of occurrences)		
	Both	MZ	DZ
Heritability shown			
Actinomyces naeslundii (−)[b]	1.05 (27)	0.71 (13)	0.33 (14)
Actinomyces naeslundii II	0.10 (8)	0.50 (3)	0.19 (5)
Actinomyces naeslundii III	0.43 (24)	0.45 (7)	0.72 (17)
Capnocytophaga gingivalis	0.56 (25)	0.38 (10)	0.06 (15)
Capnocytophaga sputigina	1.85 (13)	0.79 (6)	0.64 (7)
Eubacterium "D06"	0.98 (4)	1.00 (2)	1.00 (2)
Gemella morbillorum	0.28 (16)	0.40 (8)	0.21 (8)
Lactobacillus rimae	0.16 (22)	0.20 (11)	0.16 (11)
Neisseria mucosa	0.27 (16)	0.63 (8)	0.47 (8)
Prevotella oris	1.29 (11)	0.57 (6)	0.16 (5)
Streptococcus intermedius	0.58 (29)	0.45 (12)	0.40 (17)
No heritability shown			
Streptococcus mutans	−0.06 (11)	−0.19 (4)	−0.07 (7)
Propionibacterium acnes	−1.07 (9)	−0.37 (6)	−0.12 (3)
Staphylococcus epidermidis	−0.24 (5)	−0.12 (3)	−0.06 (2)
Mean for all species	0.02 (96[c])	0.14 (76[c])	0.17 (77[c])

[a] Both, MZ and DZ analyzed simultaneously by least-squares analysis. DZ and MZ, analysis of variance separately, where the DZ analysis is an estimate of heritability in the narrow sense.
[b] See Table 6, footnote *b*.
[c] Number of taxa that were detected in samples from two or more subjects.

culture of anaerobes and facultative microorganisms or that neither of these two species was present in any of the 22 samples cultured. Delaney et al. sampled only females, but we know from our previous studies that *A. israelii* and *A. gerencseriae* occur in females as well as in males. It is possible that in the study by Delaney et al. strains of *A. israelii* and *A. gerencseriae*, which generally are catalase negative, were identified as *A. naeslundii*. Gusberti et al. (9) reported that the frequency of detecting *A. odontolyticus* significantly increased with time in both sexes, which we did not see.

The situation regarding the association of black-pigmented bacteroides with the flora of adults rather than of children still is not clarified definitively. In the mid-1960s, Bailit et al. (2) and Kelstrup (16) reported an increasing prevalence of gingival *P. melaninogenica* with age. At that time all of the pigmented bacteroides would have been considered *B. melaninogenicus*. Inasmuch as *Prophyromonas gingivalis* rarely is isolated from children, it is reasonable to assume that most of the pigmenting strains observed by Bailit et al. and Kelstrup were either *P. intermedia* or *P. melaninogenica*. From the report of Kornman and Loesche (20), one would have expected an increase of black-pigmented bacteroides with physical maturation in females, but this was not observed by Yanover and Ellen (36). On the other hand, Delaney et al. (4) reported that the percentage of saccharolytic (mostly *P. intermedia*) black-pigmented bacteroides species in the subgingival plaque of females was higher in 6 subjects who were soon to experience menarche, as compared with the percentage recovered from 6 sexually more immature females or from 10 more mature females studied. Our results are most like those of Wojcicki et al. (35), who reported significantly higher proportions of *P. denticola/P. loescheii* and *P. melaninogenica* in postpubertal individuals (males and females) than in the younger groups. Gusberti et al. (9) also found a statistically significant rise in the frequency of *P. intermedia* and *P. melaninogenica* at the time of the onset of testicular growth in boys. Thus, our results confirm previous indications that the changes in microbial floras of individuals occur during puberty.

Because we felt that an uneven number of samples per person might skew the statistical results, the floras of previously studied children and adults with naturally occurring gingivitis were reanalyzed by using only two samples per person and compared with each other and with the floras of the 11- and 14-year-old twins. These results were similar to those previously reported (24) with all samples per person included in the analysis. Also, as would be expected, the floras of children with naturally occurring gingivitis and the floras of 11-year-old twins were not significantly different. The significant differences (Table 5) between the 11-year-old twins and the adults and the 14-year-old twins and both children and adults suggest that the flora of the 14-year-olds no longer represents that of children but does not yet represent that of adults (in parallel with their testosterone levels).

The species that appeared to be particularly associated with the teenagers (Table 6) are *V. atypica* and *Lactobacillus rimae*, whereas a variety of species, including *Bacteroides forsythus* and *Wolinella* "X," appeared to be associated with the adults.

TABLE 9. Estimates showing heritability by each of three tests at age 12.5

Taxon	Heritability value[a] (no. of occurrences)		
	Both	MZ	DZ
Actinomyces naeslundii (−)[b]	1.50 (15)	0.54 (9)	0.03 (6)
Actinomyces "NV"[c]	0.15 (20)	0.20 (11)	0.20 (9)
Bacteroides gracilis	0.27 (21)	0.42 (11)	0.35 (10)
Capnocytophaga gingivalis	0.76 (26)	0.65 (13)	0.22 (13)
Capnocytophaga sputigena	1.27 (13)	0.52 (7)	0.05 (6)
Eikenella corrodens	0.49 (7)	0.59 (4)	0.59 (3)
Lactobacillus "D10"	1.29 (10)	0.84 (6)	0.31 (4)
Prevotella intermedia 8944	0.64 (16)	0.40 (6)	0.02 (10)
Prevotella oris	0.19 (12)	0.25 (7)	0.15 (5)
Selenomonas flueggeii	0.55 (10)	0.63 (4)	0.24 (6)
Streptococcus intermedius	0.90 (24)	0.42 (13)	0.05 (11)
Mean for all species	0.23 (113[d])	0.17 (96[d])	0.08 (72[d])

[a] See Table 8, footnote *a*.
[b] See Table 6, footnote *b*.
[c] Reacted with both *A. naeslundii* and *A. viscosus* conjugates.
[d] See Table 8, footnote *c*.

TABLE 10. Estimates showing heritability by each of three tests at age 14

Taxon	Heritability value[a] (no. of occurrences)		
	Both	MZ	DZ
Actinomyces gerencseriae	0.67 (14)	0.47 (12)	0.98 (2)
Actinomyces naeslundii III	0.02 (25)	0.31 (15)	0.23 (10)
Actinomyces viscosus II	0.93 (10)	0.47 (7)	0.45 (3)
Bacteroides gracilis	0.62 (21)	0.42 (14)	0.01 (7)
Fusobacterium nucleatum	0.22 (30)	0.62 (17)	0.37 (13)
Leptotrichia buccalis	0.32 (12)	0.41 (7)	0.08 (5)
Streptococcus parvulus	0.30 (20)	0.33 (15)	0.04 (5)
Mean for all species	−0.09 (105[b])	0.15 (90[b])	0.22 (64[b])

[a] See Table 8, footnote *a*.
[b] See Table 8, footnote *c*.

The significantly greater similarity of the compositions of the floras of MZ and DZ twins compared with floras of unrelated people at most of the times tested (Table 7) indicates that genetic and environmental influences on composition of the periodontal flora may be estimated. The greater numerical similarity of the floras of MZ twins compared with those of DZ twins at ages 11, 12.5, and 14 suggests that genetic factors may influence composition of floras. The reason that the floras of the DZ twins at 12.5 years of age were not significantly more similar than those of unrelated people is not immediately apparent. It is possible that the intermediate and variable levels of testosterone within DZ twin pairs at this age were responsible for differences in the compositions of the floras and low similarity values. With a single exception in MZ twins (pair 4 at 14 years), the testosterone levels differed most in DZ twins at 12.5 and 14 years of age (mean difference, 46 and 95 ng/dl of blood, respectively).

The single major discrepancy in testosterone levels of MZ twins occurred in twin pair 4 at 14 years of age. Twin 4b had been diagnosed with leukemia, which may explain the difference between these two MZ subjects. However, it did not appear to affect the similarity of the floras in this twin pair, because the compositions of their floras were more similar than the average for MZ twins at 14 years of age (data not shown).

The heritability analyses used here generally are expected to yield values of between 0 and 1 but can yield higher or lower values because of sampling variation. Although 10,600 bacterial isolates were characterized and identified to the species level, these were divided among 238 different taxa. Thus, detection of individual species was relatively limited, and the variance for each was high. Variance also might have been increased as a result of differences in testosterone levels within DZ twin pairs at ages 12.5 and 14. At age 14 the subjects represented the entire range of maturity from prepubertal to adult, with considerable difference in testosterone levels within several of the twin pairs.

Although the present estimates are tentative, they present some of the first direct evidence that the composition of the human flora may be influenced by genetic factors. The finding that certain species appear to be controlled primarily by environmental factors lends further credibility to the data because both *Propionibacterium acnes* and *Staphylococcus epidermidis* (Table 8) are members of the skin flora and often simply represent contaminants of samples from other body sites.

Because the floras of twins are significantly more similar than those of unrelated people at both 11 and 14 years of age when they share similar environments, it is important to determine whether or not the greater similarity persists in adult twins. Possibly, different factors control the compositions of prepubertal and adult floras, or the observed genetic influence may be modified or overcome by environmental pressures in adults. A study encompassing a much larger number of adult twins is in progress.

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