Season, Sun, Sex, and Cervical Cancer

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Abstract

Introduction: **Sunlight's UV B component, a known cellular immunosupressant, carcinogen, and activator of viral infections, is generally seasonally available. Venereal human papillomavirus (HPV) transmission, at least in part, causes cervical cancer. We have previously inspected the monthly rates of venereal HPV infection and sunlight fluency in Southern Holland over 16 consecutive years. Both peak in August with at least 2-fold seasonality. The amount of available sunlight and the rate of Papanicolaou (Pap) smear screen–detected HPV are positively correlated. We now investigate whether premalignant and malignant cervical epithelial changes are also seasonal and related to seasonal sunlight fluency.**

Methods: **We have studied >900,000 consecutive, serially independent, interpretable screening Pap smears obtained by a single cervical cancer screening laboratory in Leiden, Holland, during a continuous 16-year span from 1983 through 1998. The average monthly rates of premalignant and malignant epithelial change were inspected and the annual patterns contrasted to the annual pattern of sunlight fluency at this global location and to monthly average HPV infection rate. Because HPV is venereally transmitted, Dutch seasonal sexual behavior was evaluated by assessment of the annual pattern of Dutch conception frequency as a competing cause for cervical cancer seasonality.**

Results: **(a) Twice as many premalignant and malignant epithelial changes were found among Pap smears obtained in the summer months, with an August peak concurrent with histopathologic evidence of HPV infection and sunlight fluency in Southern Holland. (b) Monthly sunlight fluency is correlated positively with both the monthly rates of Pap smear–detected cervical epithelial dysplasia and carcinomatous histopathology, as well as HPV. (c) Conception frequency, in this location, peaks in Spring not summer, and has a 4.8% annual amplitude.**

Conclusions: **(a) Cervical epithelial HPV infection and HPV-induced cervical epithelial dysplasia and carcinomatous change may each be novel sun exposure risks and thereby behaviorably avoidable. (b) Because screening Pap smears uncover many abnormalities that resolve spontaneously (false positives), these data may argue for screening and follow-up Pap smear examinations in seasons other than summer in the Northern Hemisphere, to diminish the false-positive smear rate. Global data are available to confirm and further test each of these conclusions.** (Cancer Epidemiol Biomarkers Prev 2005;14(8):1940–7)

Introduction

The largely predictable annual angular relationship of the surfaces of earth and sun, and to a lesser extent, the distance between these bodies, have imprinted genomic plans for successfully coping with the resultant seasonality. Direct solar energy availability and climate change occurring largely secondary to that annual sunlight fluency rhythm each have ongoing biological effects as well as genomic consequences. Seasonal genomic programs include a fabric of interacting molecular, cellular, organismic, and ecosystem clocks that measure and coordinate biological and environmental time, continually adjusting biological circadian/daily and circannual/seasonal time structures to the environment. This annual chronobiology permits organisms to successfully anticipate rhythmic environmental change and resonate with the seasons. Circannual rhythms powerfully affect biological function including susceptibility to infectious, metabolic, inflammatory, and malignant diseases (1, 2).

The frequency of diagnosis of many cancers including testis, prostate, and breast are nonrandomly distributed throughout each year (3, 4). Breast cancer discovery (5-8) and lethality (9-11) are each seasonal, as is its histopathologic, biochemical, and molecular aggressiveness (12-14). Some cancers, such as melanoma and squamous and basal cell skin cancers, are directly caused by the molecular genetic effects of exposure to sunlight's UV B wavelength (15, 16). Others, for example cervical cancer, are caused by viral infection; in this case, specific serotypes of the human papillomavirus (HPV; ref. 17). UV B activates many viruses both *in vitro* and *in vivo*, including HPV (15). The development of cancer always depends upon the balance between the inciting agent(s) and the competence of natural cellular immune defenses (18). UV B also suppresses cellular immune defenses against viruses and cancer both locally at the site of sun exposure and systemically (16).

We previously found that the frequency of screening Papanicolaou (Pap) smear–detected cervical epithelial HPV infection is seasonal in Southern Holland. Twice as many HPV-infected Pap smears are discovered in summer as opposed to winter, with an annual August peak (19). Because HPV is venereally transmitted, we also examined the annual pattern of conception in the Dutch population. This yearly rhythm peaks in March and has a amplitude of 4.8% (20, 21). Sunlight fluency, however, has a >2-fold annual rhythm that peaks in August as does the frequency of Pap smear–detected HPV infection. The sunnier the month (n = 192), the greater the rate of HPV, and the sunnier the year (n = 16), the greater the HPV detection rate. We now determine whether precancerous and cancerous cervical epithelial changes are also annually

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Table 1. Seasonal changes in the monthly incidence (cases/thousand/month) of Pap smears detected uterine cervical abnormalities and sunshine duration in Southern Holland (1983-1998)

Cervical abnormality	Type	Month						
		Jan	Feb	Mar	Apr	May	Jun	Jul
Dysplasia	Mild	9.64 (0.64)	9.55 (0.73)	9.67 (0.78)	9.59 (0.90)	9.69 (0.90)	**9.81** (0.80)	**11.97** (0.76)
	Moderate	3.36 (0.43)	3.11 (0.38)	2.78 (0.35)	3.19 (0.33)	3.73 (0.39)	**3.39** (0.38)	**4.69** (0.41)
	Severe	2.14 (0.26)	2.14 (0.26)	2.07 (0.21)	2.15 (0.29)	2.23 (0.30)	2.25 (0.27)	**2.87** (0.31)
	All	15.15 (1.02)	14.80 (0.95)	14.52 (1.10)	14.92 (1.12)	15.64 (1.36)	15.45 (1.26)	**19.53** (1.22)
Atypia	Mild	0.70 (0.13)	1.13 (0.09)	1.19 (0.17)	1.35 (0.22)	1.31 (0.10)	1.25 (0.20)	**2.33** (0.38)
	Moderate	1.23 (0.23)	0.69 (0.09)	0.59 (0.10)	0.61 (0.14)	0.89 (0.12)	0.60 (0.10)	**1.22** (0.28)
	Severe	1.58 (0.13)	1.61 (0.14)	1.22 (0.21)	**1.86** (0.29)	1.51 (0.20)	1.15 (0.14)	1.60 (0.23)
	All	3.51 (0.34)	3.43 (0.20)	3.00 (0.36)	3.82 (0.44)	3.72 (0.26)	3.00 (0.35)	**5.16** (0.69)
Carcinoma	*In situ*	0.90 (0.18)	1.04 (0.21)	1.01 (0.11)	1.22 (0.17)	1.12 (0.16)	1.11 (0.16)	**1.42** (0.20)
	Microinvasive	0.05 (0.03)	0.05 (0.03)	**0.12** (0.04)	0.10 (0.05)	0.13 (0.06)	0.05 (0.03)	0.10 (0.04)
	Frankly invasive	0.10 (0.05)	0.11 (0.04)	0.11 (0.04)	0.09 (0.03)	**0.20** (0.04)	0.10 (0.04)	0.12 (0.05)
	All	1.05 (0.22)	1.20 (0.24)	1.24 (0.15)	1.41 (0.19)	1.45 (0.18)	1.26 (0.17)	**1.64** (0.23)
Venerial/viral infection		4.74 (0.49)	4.66 (0.65)	4.91 (0.76)	5.02 (0.56)	5.00 (0.63)	5.64 (0.91)	**7.13** (1.20)
% Maximum sunshine duration†		21.69 (2.06)	30.16 (1.65)	33.56 (2.39)	41.61 (2.60)	**43.88** (3.61)	40.6 (2.32)	**45.67** (1.95)

NOTE: Values are mean (SE). Means in bold correspond to highest values.
Abbreviation: ROC, range of change from minimum to maximum monthly average (% minimum monthly value).
*Single cosinor = least-squares fit of a 12-month cosine to 198 values from 1983 to 1991, except for all cases of atypia with 108 values from 1983 to 1991.
†Percentage of maximum possible sunshine duration.

rhythmic and whether their annual patterns are similar to and correlated with the annual sunlight fluency and HPV patterns in this same global location over these same 16 consecutive years (18).

Materials and Methods

Every interpretable Pap smear referred to the Leiden Laboratory over 192 consecutive months between 1983 and 1998, as part of the Dutch National Cervical Cancer Screening Program, has been included in this analysis (22). This catchment area represents one third of the adult female population of southern Holland. All women with discovered abnormalities are eliminated from further screening, reported to their respective gynecologist, and followed up elsewhere in the Netherlands; the recommended frequency of screening Pap smears is once every 4 to 5 years. Therefore, these Pap smears are virtually serially independent samples. Both urban and rural women of all adult ages are fully represented in this population. During these 16 years, 920,359 smears were processed, read, and reported by the same expert technical staff, and each abnormal smear was reviewed by the same pathologist and laboratory director (M.E.B.). Between 1983 and 1991 (504,093 smears) epithelial abnormalities were reported as mild, moderate or severe dysplasia or atypia (six categories) or as carcinoma *in situ*, microinvasive carcinoma, or frankly invasive cervical cancer (three additional categories). After 1991 (416,266 smears), the three atypia categories were abandoned and all significant epithelial abnormalities were folded into six rather than nine categories. Throughout the entire 16-year span, the koilocytic and multinucleate cytologic footprints of venereal HPV epithelial infection were consistently identified and recorded (22).

Brief Definitions of Described Histopathologic Abnormalities. Over the 16 years of this study, three terms have been used in this screening lab for histopathologically abnormal uterine epithelium: atypia, dysplasia, and carcinoma *in situ*. Atypia in this Leiden laboratory is equivalent to borderline malignant change in the United Kingdom and atypical cells of unknown significance in the United States. This is the first and least abnormal identifiable state along the histopathologic path toward cancer. Dysplasia is the same as dyskariosis in the United Kingdom and a squamous intraepithelial lesion in the United States. This abnormality shows nuclear and cytoplasmic changes more severe than atypia. This lesion is often graded from least to most abnormal (D1, D2, and D3) across the degrees of abnormality. Carcinoma *in situ* is the most severe form of dysplasia, worse than D3 but still not frankly invasive.

Sunlight Data. Sunlight data were collected four to six times daily at Vlissingen, Zeeland province by the Royal Netherlands Meteorological Institute (23). The percentage of maximum possible sunshine duration was averaged for each of the 192 months between 1983 and 1998.

Data Analysis. On the average, some 4,791 Pap smears were examined monthly throughout these 16 years. Significantly fewer smears were, however, examined in the months of July (3,990), August (3,109), and January (4,241). All data were therefore normalized before analysis by expressing the rates of each abnormality detected per thousand smears inspected during that month. All 192 consecutive normalized monthly rates of Pap smear abnormality and monthly average percentage of possible maximum sunshine were subjected to three types of ''time series'' analysis: descriptive, parametric (ANOVA), and nonparametric (single and harmonic cosine function fit) to describe the shape of recurring patterns of these data across the year. In addition, relational analyses were accomplished using the Pearson correlation test.

Descriptive Analyses. Pictorial descriptions of the 192 monthly rates of cervical abnormality and corresponding monthly sunlight availability across 16 consecutive years were graphed. These pictures show consistent and cumulative seasonal differences in these rates during each year. The range of change is expressed as the average monthly minimum to maximum difference, as percentage of that minimum monthly value. This gives an idea of the relative amount of within year minimum to maximum range of monthly change of the specific abnormalities.

ANOVA. Twelve monthly rates of each abnormality are recorded across each of 16 years (192 data points). If these rates had no consistent pattern across time, then a one-way ANOVA of each of 16 repeat monthly rates across the 12 months of the

Table 1. Seasonal changes in the monthly incidence (cases/thousand/month) of Pap smears detected uterine cervical abnormalities and sunshine duration in Southern Holland (1983-1998) (cont'd)

Aug	Sep	Oct	Nov	Dec	Overall mean	ROC (%)	ANOVA		Cosinor analysis*			
							Fc	*P*	*P*	Acrophase	95% confidence interval	
12.97 (1.14)	**10.68** (0.92)	9.94 (0.61)	8.19 (0.67)	9.36 (0.67)	10.09 (0.24)	58	2.45	0.0071	0.002	July 25	June 21	Aug 29
4.49 (0.55)	**3.36** (0.34)	3.00 (0.28)	2.53 (0.37)	2.62 (0.28)	3.35 (0.12)	85	3.15	0.0006	<0.001	July 8	June 11	Aug 4
3.38 (0.48)	**2.56** (0.33)	2.35 (0.19)	1.82 (0.22)	2.14 (0.16)	2.34 (0.08)	85	2.17	0.0180	0.002	Aug 3	June 29	Sep 7
20.84 (1.81)	**16.60** (1.31)	15.29 (0.87)	12.54 (1.01)	14.11 (0.94)	15.78 (0.37)	66	3.7	0.0001	<0.001	July 22	June 25	Aug 17
2.01 (0.25)	**1.58** (0.22)	1.00 (0.12)	1.40 (0.26)	1.32 (0.17)	1.38 (0.06)	233	2.44	0.0098	0.002	July 26	June 23	Aug 29
1.88 (0.81)	0.60 (0.10)	0.69 (0.11)	0.43 (0.08)	0.87 (0.13)	0.86 (0.08)	338	1.25	0.2665	0.477	Aug 6	—	—
2.50 (0.59)	**1.62** (0.23)	1.61 (0.09)	1.42 (0.12)	1.67 (0.17)	1.61 (0.07)	118	1.07	0.3929	0.516	Sep 4	—	—
6.40 (1.01)	3.80 (0.25)	3.31 (0.19)	3.25 (0.35)	3.86 (0.22)	3.86 (0.14)	114	2.67	0.0050	0.020	Aug 6	June 22	Sep 20
1.78 (0.23)	**1.54** (0.22)	0.97 (0.13)	0.81 (0.16)	0.78 (0.15)	1.14 (0.05)	127	2.88	0.0017	<0.001	July 18	June 20	Aug 16
0.11 (0.06)	**0.13** (0.05)	0.06 (0.03)	0.07 (0.03)	0.05 (0.03)	0.09 (0.01)	179	0.70	0.7402	0.331	June 19	—	—
0.15 (0.07)	0.16 (0.05)	0.08 (0.05)	**0.19** (0.05)	**0.19** (0.06)	0.13 (0.01)	145	0.78	0.6635	0.724	Oct 5	—	—
2.05 (0.30)	**1.83** (0.24)	1.12 (0.14)	1.07 (0.19)	1.02 (0.18)	1.36 (0.06)	101	2.51	0.0058	<0.001	July 19	June 19	Aug 18
8.46 (1.21)	**6.22** (0.77)	6.17 (0.77)	4.27 (0.47)	4.80 (0.56)	5.59 (0.24)	98	2.4	0.0080	0.001	Aug 4	July 7	Sep 1
46.75 (2.21)	36.64 (2.04)	34.42 (1.89)	24.4 (1.94)	18.46 (1.77)	34.82 (0.91)	153	17.8	<0.0001	<0.001	June 11	May 11	July 12

year would not yield statistically significant nonrandom distribution of the variance across the overall monthly means. These *F* and *P* values are presented under the table heading ANOVA for each abnormality, as well as sunlight availability (Table 1).

Cosinor Analysis. This form of time series analysis asks a simple question: Is the shape of the data sinusoidal and similar from year to year rather than either random or simply trending across the entire observation span? The procedure used is entirely analogous to the one used to define a linear trend in data (i.e., by testing the fit of the best-fitting straight line to those data and subsequently determining the probability that the slope of this best-fit line is different from zero). The method for determining the best fit of any straight line is to sum the squares of all the distances of each data point from every possible straight line. The line with the smallest ''sum of squares'' is the best-fit line. The cosinor method is identical, but instead of a straight line, the model fits a cosine function described by a mean, an acrophase, and an amplitude. The mean is called the mesor. The series mesor and mean are identical if the data are equidistant across the sampling span but are not identical if sampling is irregular because the model weights the values by assuming sinusoidality across time. The 192 monthly data analyzed here are equidistant across the 12 calendar months. The acrophase is the time from a phase reference to the peak of the cosine function best describing the data. For annual analyses, this phase reference is, by convention, midnight of January 1. The amplitude is the height of the best-fitting cosine function, from the mesor/mean to the acrophase. This is one half of the full variation from trough to peak of that cosine function. The probability that the amplitude of the cosine function best fitting these data is >0 is calculated based upon the quality of the cosine fit. If the zero amplitude hypothesis must be rejected, the fit is statistically significant ($P \leq 0.05$).

Cosinor analysis can also be used to fit more than one ''harmonic,'' the concurrent fit of functions with other periods. Because our hypothesis was that an annual rhythm exists, the 8,766 hours/1-year period was invariably fit to each data series. Concurrent fitting of 4,383 hours/6-month period and 2,922 hours/4-month periods sometimes enhanced the fit of the circannual component. Multiple-component linear least squares analysis allows rejection of the zero amplitude hypothesis for each individual component rhythm tested, concurrently providing amplitudes and acrophases for each period fit to these data. In the case of the data series described in this article, fitting the annual period plus one subharmonic at a 6-month rhythm enhances the quality of curve fit for some end points. If one peak of the two component fit is higher, a major and minor component are each present in the data. In this case, the major peak (the highest acrophase) is termed the orthophase of a multiphase-component cosine function fit (24-26).

Correlative Analyses. The relationship among the rates of various histopathologic, cervical epithelial abnormalities, and sunlight availability was further investigated by correlating the annual amplitude of these changes (winter-summer differences) with the annual amplitude of sunlight availability. If sunlight is an important driver of cervical pathology, one might expect larger winter-summer differences in these rates of abnormalities in years where winter-summer differences in sunlight are also most profound. Correlation analysis was used to relate these two things over the 16 years for which both sets of data are available. Monthly averages were correlated to determine whether months with higher frequency of precancerous and cancerous abnormalities coincide to the months with higher sunshine availability. Likewise, to establish that such a relationship exists year to year, annual monthly maximum observed sunlight availability and maximum monthly frequency of cervical abnormalities were correlated. Three-way correlations were also done among average monthly sunlight fluency, HPV, and dysplasia; sunlight fluency, HPV infection, and carcinomatous changes; and sunlight fluency and dysplastic and carcinomatous changes.

Results

Table 1 lists the monthly mean rate of every type of examined epithelial abnormality: mild, moderate, severe, and all dysplasia (1983-1998); mild, moderate severe, and all atypia (1983-1991); *in situ*, microinvasive, frankly invasive carcinoma, and all cancerous abnormalities (1983-1998); as well as cytologic evidence of venereal HPV infection of the cervical epithelium (1983-1998); sunlight fluency in this geographic area (1983-1998). ANOVA of these normalized rates, across the year for each of these 192 monthly observations, shows the nonrandom distribution of virtually every general category of epithelial abnormality evaluated. The range of change across each year varies from 58% to 338% and the average range of yearly change is sometimes ≥2-fold for some abnormalities. Cosinor analysis of these same rate data reveal statistically significant reproducible annual patterns in each dysplasic state, mild atypia, carcinoma *in situ*, as well as the cytologic footprints of HPV infection within the cervical epithelium. Each of the combined categories (all dysplasias, all atypias, and all carcinomas) shows

highly statistically significant, stable, and reproducible annual patterns. The peaks of the cosine functions best describing each of these data series are overlapping and overlap with the 95% confidence interval of the annual peak of sunlight fluency.

To visually show whether these patterns are the result of a few years with very high summer incidences or a year-to-year consistent pattern and to illustrate the year by year peak-trough differences of these abnormalities and sunshine availability, ''layer cake'' cumulative frequency diagrams were constructed (Fig. 1). For the abnormalities, the monthly data in each figure are expressed as cumulative 16-year rates (per 1,000 smears). The average annualized monthly rate is calculated by dividing this number by the 16 years of observation. Figure 1A shows monthly sunlight fluency for each of these 192 months averaged by year. Figure 1B shows the year-to-year pattern of the monthly rates of all dysplasias (1983-1998), with a peak-trough cumulative frequency difference between 200 and 325 occurrences/1,000 screenings/mo (over 16 years). Figure 1C shows cumulative frequencies of all atypias (1983-1992 only). The summer peak is obvious, with an annual range of between 28 and 58 monthly cumulative abnormalities, roughly 2-fold. Figure 1D shows the 16-year cumulative monthly pattern of all carcinomatous readings (*in situ*, microinvasive carcinoma, and frankly invasive carcinoma). The monthly range of these critical events varies about 2-fold from 16 to 33 events per 1,000 monthly smears. This 2-fold difference represents the discovery of two cancers rather than 1 per 1,000 smears/mo in summer compared with winter. Figure 1E shows the cumulative annual frequencies of venereal HPV infection. Over the entire span (1983-1998) and during each year, histopathologic evidence of cervical epithelial viral infection is about twice as frequent in the summer months. Together, these results indicate that putative viral precursor lesions and all grades of premalignant and malignant cervical epithelial change are discovered, in southern Holland, at least twice as frequently in summer than winter months. Figure 2 overlies the average monthly sunlight pattern and the patterns of each cervical epithelial abnormality (atypias, dysplasias, carcinoma, and HPV). The patterns of each of these abnormalities peak in August and have very similar slopes, rising steadily and decreasing rapidly. The sunlight fluency pattern also peaks in August but increases earlier and more gradually then decreases off rapidly as do the other time structures.

Relationship of Annual Sunlight Fluency and Cervical Abnormalities. Over these 16 years, and for most individual years, the percentage of possible sunlight was highest in the month of August ($F = 17.8$, $P < 0.001$, Table 1). The 95% confidence interval of the cosine function best fitting these sunlight data overlaps that of many of the cervical abnormalities (Table 1). The relationship between the amount of sunlight and the rate of cervical abnormality, over these 16 years, reveals that the greater the yearly summer sunlight availability, the greater the annual average frequency of dysplasia ($r = 0.56$, $P = 0.02$), frankly invasive carcinoma ($r = 0.65$, $P = 0.01$), and cervical epithelial HPV-associated changes ($r = 0.59$, $P = 0.01$). To further investigate the association between sunlight and monthly epithelial abnormalities, the monthly average sunshine fluency were correlated over the 192 months of observation. Average monthly sunshine duration is significantly correlated to average monthly observed frequency of dysplasia ($r = 0.71$, $P = 0.001$), all carcinoma ($r = 0.76$, $P = 0.004$), and HPV ($r = 0.67$, $P = 0.016$; Fig. 3).

Three-Way Interactions. Because sunlight, which is positively associated with HPV, may, in principle, affect the progression of epithelial changes to dysplasia and cancer by similar mechanisms, the three-way relationships were examined and quantified by computing partial correlations among (a) sunlight, HPV, and dysplasia; (b) sunlight, HPV, and carcinoma; and (c)sunlight, dysplasia, and carcinoma.

Relationships Among Sunlight Duration, HPV Discovery, and Dysplastic Epithelial Change. Over 16 consecutive years of observation (1983-1998), monthly average sunlight duration correlates positively with both the monthly average frequency of HPV infected screening Pap smears and the monthly average frequency of dysplastic screening Pap smears ($R^2 = 0.9$, $P < 0.01$, Fig. 4). The marginal component correlations reveal that the greater the monthly average sunlight duration, the greater the monthly average frequency of HPV-infected smears ($r = 0.61$, $P = 0.04$). Sunlight duration is positively but less strongly correlated with dysplastic smear frequency ($r = 0.41$).

Relationships Among Sunlight Duration, HPV Detection, and Carcinomatous Epithelial Change. Over 16 consecutive years of observation (1983-1998), monthly average sunlight duration correlates positively with both the monthly average frequency of HPV infected screening Pap smears and the monthly average frequency of carcinomatous screening Pap smears ($R^2 = 0.76$, $P < 0.01$, Fig. 5). The component correlations reveal a weak positive relationship between monthly average sunlight duration and the HPV frequency ($r = 0.21$). There is, however a strong positive relationship between monthly average sunlight duration and the monthly rate of cancerous epithelial change on the Pap smear ($r = 0.76$, $P < 0.01$). The relative strength of these component relationships indicates that sunlight exposure may be an important contributor to progression.

Relationships Between Sunlight Duration and Dysplastic and Carcinomatous Epithelial Change. Over 16 consecutive years of observation (1983-1998), monthly average sunlight duration correlates positively with both the monthly average rates of both dysplastic and carcinomatous cervical epithelial change found among nearly a million screening Pap smears ($R^2 = 0.78$, $P < 0.01$, Fig. 6). The greater the sunlight availability, the greater the frequency of dysplasia ($r = 0.68$, $P = 0.02$) and carcinoma ($r = 0.44$, $P = 0.17$). These relationships indicate that the progression from normal to dysplasia and, perhaps, to carcinoma may each be facilitated by sunlight.

Annual Patterns of Dutch Heterosexual Behavior. The annual pattern of conception frequency in Holland over the last several centuries has been reported. The human heterosexual behavior responsible for conception peaks near the vernal equinox and, in the most recent decades, the amplitude of this annual rhythm is 4.8% (20, 21).

Cytotechnologist Holiday Effects. One of a team of seven experienced cytotechnologists screened each smear. The vacation schedules of each of these seven technologists were inspected over 48 consecutive months to rule out the possibility that the regular absence of (a) key individual(s) in and around August could, in part, explain the August peak in cervical epithelial abnormalities. Vacations of all technologists were more common between May and September than between September and May. Visual inspection of the individual vacation blocks of each of the technicians across these 4 years revealed no obvious pattern for all or any technologist. Specifically, no individual technologist routinely took vacation in any of the weeks of August ($\chi^2 = 9.1$, $P = 0.17$). Furthermore, the vacation pattern of the entire group over these 48 months was unable to explain the August peak. The weeks during which vacation was not taken over the number of total weeks available revealed: 89.29% staffing for May, 84.3% for June, 89.29% for July, 82.14% for August, and 82.14% for September ($\chi^2 = 0.7$, $P > 0.95$).

Discussion

Mass cervical cancer screening programs in developed countries identify one in 10 young women as cytologically ''at risk'' for a disease that is likely to occur in one in many

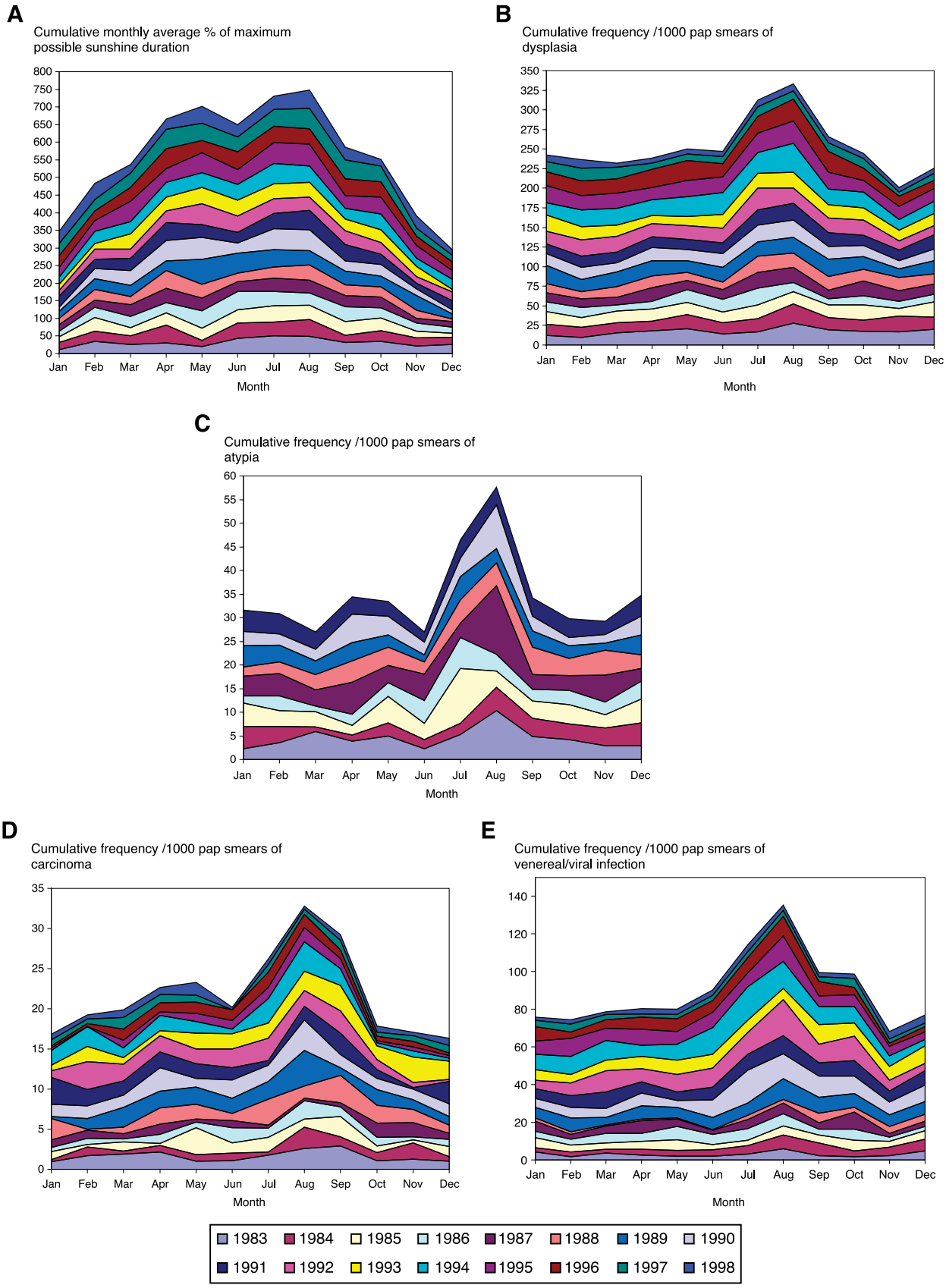

Figure 1. A. Cumulative monthly average % maximum possible sunshine duration in Southern Netherlands from 1983 to 1998. **B.** Cumulative frequency per 1,000 screenings of Pap smear (*dysplasia*) abnormalities in Southern Netherlands from 1983 to 1998. **C.** Cumulative frequency per 1,000 screenings of Pap smear (*atypia*) abnormalities in Southern Netherlands from 1983 to 1991. **D.** Cumulative frequency per 1,000 screenings of Pap smear (*carcinoma*) abnormalities in Southern Netherlands from 1983 to 1998. **E.** Cumulative frequency per 1,000 screenings of Pap smear (*venereal viral infection*) abnormalities in Southern Netherlands from 1983 to 1998.

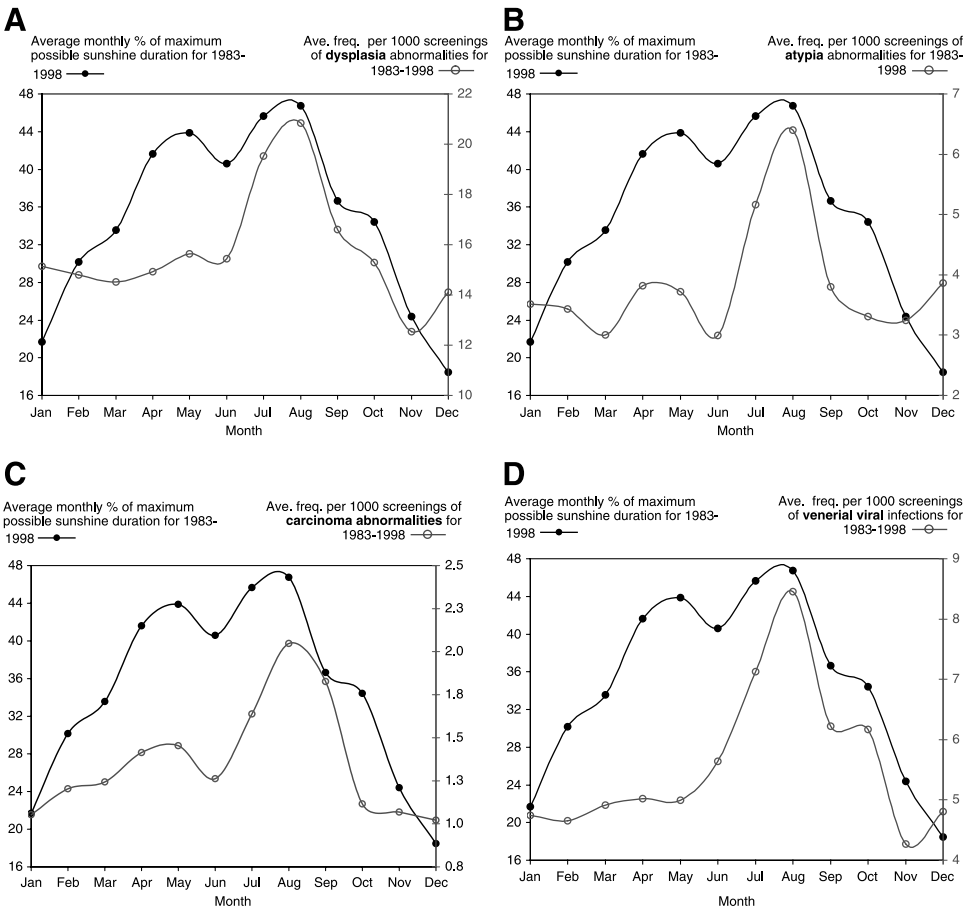

Figure 2. A. Average monthly % maximum possible sunshine duration and average monthly incidence (per 1,000 screenings) of dysplasia infection in Southern Netherlands (1983-1998). **B.** Average monthly % maximum possible sunshine duration and average monthly incidence (per 1,000 screenings) of atypia infection in Southern Netherlands (1983-1991). **C.** Average monthly % maximum possible sunshine duration and average monthly incidence (per 1,000 screenings) of carcinoma infection in Southern Netherlands (1983-1998). **D.** Average monthly % maximum possible sunshine duration and average monthly incidence (per 1,000 screenings) of venereal viral infection in Southern Netherlands (1983-1998).

thousands of them (27). Both mild and moderate cervical epithelial dysplasias are more likely to regress than to progress. Four in five severe dysplasias do not progress to carcinoma *in situ*, or worse, over a 10-year follow-up span (28, 29).

Accurate live birth records have been kept in European countries for centuries. These records document that sexual activity that results in birth 9 months later is not randomly distributed around the year. In Holland, a stable annual rhythm in conception frequency with a peak near the vernal equinox characterizes this type of sexual activity. The amplitude of this annual rhythm is currently 4.8% of the mean rate. Whereas it is entirely possible that this low-amplitude spring peaking rhythm in sexual activity can help explain the annual time structure of this venerally transmitted malignancy, we thought that there might be other cause and thereby investigated the annual pattern of Dutch population ultra violet radiation exposure in Southern Holland. Because of the seasonality of other human cancers and human conception, because HPV is activated and cellular immunity is depressed by sunlight, and because sunlight fluency is seasonal, we hypothesized that cervical epithelial cancerous changes are also seasonal and phase-locked to the annual pattern of sunlight fluency. In 1987, we reviewed 144,018 serially independent screening Pap smears obtained over a little more than two consecutive years between October 1982 and May 1985 by the Leiden Cytology and Pathology Laboratory (Holland) and found that the frequency of dysplastic cervical epithelial changes seemed higher in summer (30). We have recently shown that sunlight fluency and seasonal HPV infection are associated, both peaking in August (19). This article relates the annual pattern of (population) sunlight exposure, cervical epithelial HPV discovery, and the Pap smear discovery of all grades of premalignant and frank malignant change.

Because many have thought that cervical epithelial premalignant changes progress in a stepwise fashion over many months or years, we initially expected a stepwise annual time structure with the most malignant changes appearing later in the year than the less severe changes. Because, however, it is becoming evident that cervical epithelial changes reverse more frequently than they progress (28), we also considered the possibility that if an external event such as sun exposure peaking at the same fixed time each year both causes malignant progression and diminishes spontaneous regression of malignant changes. In this case, we might alternatively expect a similar seasonality of each stage of malignant change. This synchronicity would be caused by the concurrent incitement of progression and diminishment of regression by this external (UV B mediated) stimulus, at that same time each year. These seasonal regression dynamics are especially important because of the fact that four of five of even the most abnormal epithelial changes spontaneously regress (29).

In fact, similarly timed 2-fold August peaks were found in all premalignant and malignant cervical epithelial characteristics across each of the 16 years of observation. These peaks occurred concurrent with the August peak in sunlight availability and cervical epithelial HPV associated changes. Strong positive relationships were detected between monthly sunlight fluency and each of these monthly rates of malignant change across these 192 months of observation. The similar temporal patterns of HPV infection and all premalignant and each malignant cervical epithelial change, support the idea that some similarly timed external annual event(s) synchronize(s) the biology of cervical carcinogenesis. Sunlight availability is only one of many candidate external events, with similar annual dynamics. Sunlight, however, has known suppressive effects upon cellular immunity and proven stimulatory effects upon HPV activation and is a known cause of (melanoma, epidermoid, and basal cell) cancer (18, 31, 32).

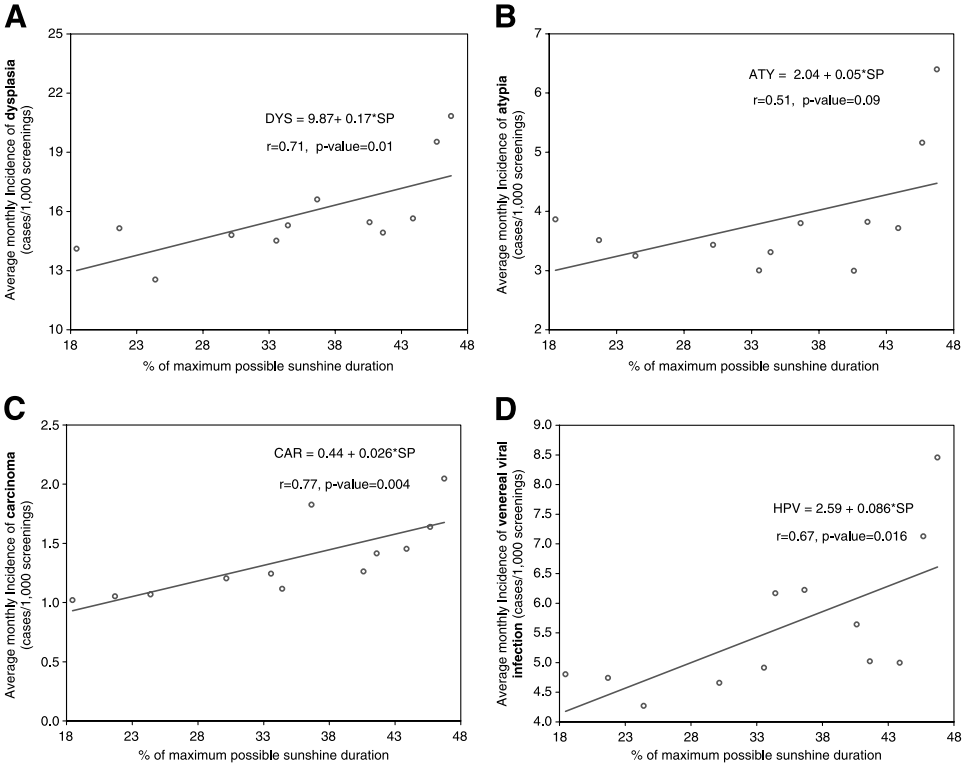

Figure 3. A. Monthly average % maximum possible sunshine duration (DYS) in a year versus average monthly incidence (per 1,000 screenings) of dysplasia infection in Southern Netherlands (1983-1998, n = 12). **B.** Monthly average % maximum possible sunshine duration in a year versus average monthly incidence (per 1,000 screenings) of atypia infection (ATY) in Southern Netherlands (1983-1991, n = 12). **C.** Monthly average % maximum possible sunshine duration in a year versus average monthly incidence (per 1,000 screenings) of carcinoma infection (CAR) in Southern Netherlands (1983-1998, n = 12). **D.** Monthly average % maximum possible sunshine duration in a year versus average monthly incidence (per 1,000 screenings) of venereal viral infections (HPV) in Southern Netherlands (1983-1998, n = 12).

We are now expanding our search for Pap smear–detected annual patterns of premalignant and malignant cervical epithelial changes and local sunlight fluency to other populations in global locations with different annual patterns of population UV B exposure. Our data indicate that if sunlight fluency contributes causally to these cervical epithelial changes, then a common annual peak in all abnormalities might be found rather than a sequential temporal seasonal pattern of least to most malignant change.

There are other less direct connections between light and cancer that may also be relevant. Light also affects pineal melatonin production and release (33, 34). This hormone has neuroendocrine effects upon human cancer (35). Day length and its rate and direction of change are translated into neurohumoral information through the pineal hormone melatonin, daily telling each cell in the body both time of day and season (36-40). Darkness associated with total blindness and robust free-running melatonin dynamics diminishes the risk of cancer (41). Conversely, shift-work and presumably the nocturnal light exposure associated with it disrupts nocturnal melatonin dynamics and increases cancer risk (42-44). The seasons do modulate nightly melatonin exposure in human beings (33, 36, 39, 45).

We conclude that the Pap smear detectability of venereal HPV infection, premalignant and malignant uterine cervical epithelial change are each rhythmic during each year at this specific location. The consistent August peak of all premalignant abnormalities argues for the metering of largely reversible susceptibility by an external synchronizer. This 2-fold annual rhythmicity may occur, at least in part, in response to annual differences in human sunlight exposure, and subsequent systemic cellular immune changes that allow HPV to integrate, activate, and transform cervical cells more effectively. The correlations of sunlight fluency with dysplastic and cancerous

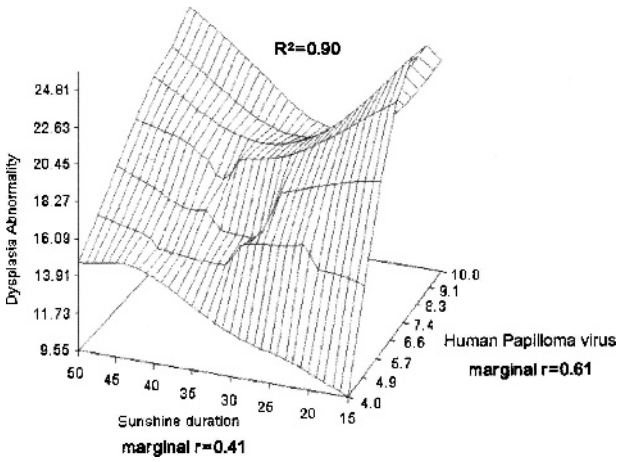

Figure 4. Sunlight duration, HPV infection, and dysplastic epithelial change in screening cervical Pap smears are positively correlated.

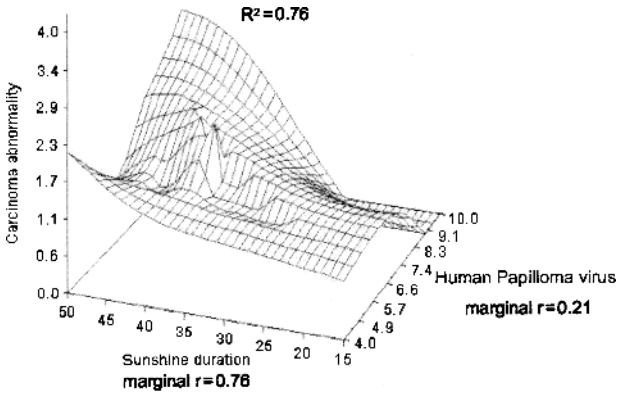

Figure 5. Sunlight duration, HPV infection, and carcinomatous epithelial change in screening cervical Pap smears are positively correlated.

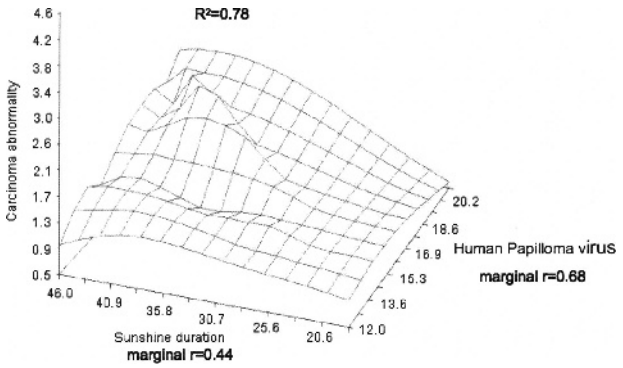

Figure 6. Sunlight duration and dysplastic and carcinomatous epithelial change in screening uterine cervical Pap smears are positively correlated.

cervical epithelial change indicate that the progression, as well as the initiation of cervical cancer, may be affected by sunlight exposure. These data also indicate that from a mass-screening perspective, the signal-to-noise ratio of the Pap smear may be predictably different in specific seasons. From an individual's point of view, the annual timing of a screening Pap smear may be critically important to whether an abnormality is discovered, and, both the date of and the interval between that first abnormal smear and the follow-up smear may, in part, determine whether the abnormality apparently resolves, persists or progresses. Perhaps, for example, regression rather than progression would have been observed if the follow-up smear had been done in any season other than summer. This is a testable hypothesis for which relevant data already exist. Finally, if sunlight exposure is responsible for these 2-fold annual differences, then sunlight avoidance may prevent human cervical cancer.

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